Does 12-weeks of exercise training reduce the risk of infertility in obese women?

A Pilot Study

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By

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ABSTRACT

Obese women face a number of health challenges, including infertility and are three times more likely to present with infertility compared to normal weight women. Exercise improves infertility by interacting with hormones specific to the reproduction process.

The hormones leptin and kisspeptin (KiSS) are crucial for reproduction. Research suggests leptin may increase production and secretion of KiSS. KiSS subsequently increases gonadotropin-releasing hormone, activating the menstrual cycle and reproduction.

Interestingly, circulating levels of leptin and KiSS are altered in the obese state. Leptin levels significantly increase, whereas KiSS levels decrease. Studies demonstrate increases in leptin cause leptin resistance, which is followed by a decrease in KiSS mRNA concentrations.

Exercise is associated with decreases in circulating leptin and thus an association with infertility, however there is no research examining the changes in KiSS after an exercise intervention. Therefore the purpose of this research was to examine the effects of an exercise intervention on circulating KiSS and leptin levels in obese women.

Ten obese women were randomized to an exercise intervention (n=5) or a non-exercise control group (n=5). The exercise intervention group completed a 12-week supervised, progressive, aerobic exercise program that involved walking on a treadmill between 65 – 75% of their predicated maximum heart rate. The non-exercise control group maintained their current lifestyle habits. All participants had blood drawn at three different time points; baseline, mid-point and end-point; and KiSS and leptin levels were analyzed.

The exercise group had a significant decrease in percent body fat (%BF) ($p<0.01$) compared to the control group from baseline to end-point testing, whereas there was a significant increase in weight ($p<0.01$) and BMI ($p<0.01$) in the control group from baseline to mid-point testing. A group main effect for circulating leptin levels was found ($p<0.01$), however a pairwise comparison between the exercise and control group was not significant ($p=0.81$). For KiSS there

was a main effect of time ($p<0.05$). Test of Within-Subjects Contrasts indicated that there was a significant decrease in KiSS between baseline and end-point testing ($p=0.05$).

Individual participant weight, leptin and KiSS data was also graphically. Trends in the individual weight, leptin and KiSS data suggest leptin levels in the exercise group corresponded with changes in weight; as weight decreased from baseline to mid-point testing, so did leptin levels. When individual participant KiSS levels were looked at in conjunction with leptin changes over the intervention potential trends did appear. All exercise participants experienced decreases in leptin from baseline to mid-point testing and all but one exercise participant saw increases in KiSS levels during the same time frame.

Overall, results support a decrease in %BF in the exercise group compared to the control group. However, there was no evidence to support that an exercise intervention for obese women statistically significantly decreased circulating leptin levels and increases KiSS levels. Although there was a trend for hormone levels to be associated with body fat levels, the small sample size was problematic. A definitive study with increased numbers is now required to elucidate whether the trends move towards significant levels.

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First and foremost, I would like to thank my supervisor, Dr. Carol Rodgers. Her faith and trust allowed me to take on a project that I was excited for and believed in. Also, her guidance and encouragement over the past two and half years has been invaluable, I have learned more than I could have ever imagined. I would also like to thank all of my committee members, Dr. Roger Pierson, Dr. Adam Baxter-Jones and Dr. Patricia Doyle-Baker for their guidance and support throughout this project.

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Finally, I want to thank my family, Betty, Travis, and Breanna, for their continued support and encouragement. Without each of you, I would not be who I am as well as where I am today. I love you all.

DEDICATION

I would like to dedicate this work to my family because without their unwavering support, this project would not have been completed. Mom, your constant belief in me, the late night phone calls of reassurance and unending love, makes me proud of who I am and where I will go. To Breanna, you are more than a sister you are my best friend. You always know what to say and when to say it, whether it is words of encouragement or telling me to relax, you get me. Finally to Travis, it was you that gave me the first spark of inspiration and the encouragement to do this, it is because of you I even considered doing a masters. I owe all of the work I have completed over the last two and half years to your belief in me.

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LIST OF ABBREVIATIONS

ACC	Acetyl-Coenzyme A Carboxylase
ACSM	American College of Sports Medicine
α-MSH	α-melanocyte-stimulating hormone
AMPK	5’ Adenosine Monophosphate-Activated Protein Kinase
ARC	Arcuate Nucleus
ARP	Agoui-Related Peptide
ATP	Adenosine Triphosphate
BBB	Blood Brain Barrier
BIA	Bioelectrical Impedance
BMI	Body Mass Index
cAMP	Cyclic Adenosine Monophosphate
cm	Centimeters
CSEP	Canadian Society for Exercise Physiology
CSEP-CEP	Certified Exercise Physiologist
DXA	Dual Energy X-Ray Absorptiometry
E_2	Estradiol
$E_2\alpha$	Estradiol Alpha Receptors
EDTA	Ethylenediamineteraacetic acid
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
ER	Endoplasmic Reticulum
FFA	Free Fatty Acids
FM	Fat Mass
FSH	Follicle Stimulating Hormone

FP	Follicular Phase
GC	Glucocorticoids
GDP	Guanosine Diphosphate
GPCR	G-Protein Coupled Receptors
GnIR	Gonadotropin-Inhibiting Hormone
GnRH	Gonadotropin-Releasing Hormone
GnRH-R	Gonadotropin-Releasing Hormone Receptors
GTP	Guanosine Triphosphate
HbA1c	Glycated Hemoglobin test
HBP	Hexosamine Biosynthesis Pathway
HR	Heart Rate
HRmax	Heart Rate Maximum
HRR	Heart Rate Reserve
HSL	Hormone Sensitive Lipase
IL-6	Interleukin-6
IVF	In Vitro Fertilization
JAK/STAT3	Janus Kinase/Signal Transducer 3 pathway
kg	Kilogram
KiSS	Kisspeptin
KiSS-R	Kisspeptin Receptors
LH	Luteinizing Hormone
LM	Lean Mass
LP	Luteal Phase
MAPK	Mitogen-activated Protein Kinase Cascade
MCH	Melanin-Concentrating Hormone

m	Meter
mRNA	Messenger RNA
mTOR	Mammalian Target of Rapamycin Signaling Pathway
NPY	Neuropeptide Y
Ob/Ob	Leptin Deficient Mouse Model
OB-Rb	Long Form Leptin Receptor
OVX	Ovariectomized
PAC	Physical Activity Complex
PAQ-AD	Physical Activity Questionnaire for Adults
PAR-Q+	Physical Activity Readiness Questionnaire Plus
%BF	Percent Body Fat
PDE3B	Phosphodesterase 3
PIK3	Phosphoinositide 3-Kinase Signaling Pathway
PMHR	Predicted Maximal Heart Rate
POMC	Pro-opiomelanocartin
RUH	Royal University Hospital
SCOS3	Suppressor Cytokine Signaling 3
TG	Triglycerides
TNFα	Tumor Necrosis Factor-α
UDP-GlcNAc	Uridine-5’-diphosphate-N-acetyglucaoslation
UPR	Unfolded Protein Response
WC	Waist Circumference
$VO2_{max}$	Voluntary Maximum Oxygen Uptake

1.0 INTRODUCTION

Obesity in women has been linked with infertility (Moran, Dodd, Niesenblat, Norman, 2011). Kisspeptin (KiSS) and leptin are hormones associated with reproductive function (Evans & Anderson, 2012). Circulating KiSS (Qunennell et al., 2011) and leptin levels (Matsubara, Maruka, Katayose, 2002) are found to be abnormal in obese states. Research has shown that exercise beneficially effects weight loss and circulating leptin levels in obese women. However the effects of exercise on circulating KiSS in obese women has yet to be studied.

1.1 Infertility

Infertility is broadly defined as the inability of a woman to conceive when a conception risk is present (Bushnik, Cook, Yuzpe, Tough, Collins, 2012). Infertility affects up to 15.7% of Canadian women and can often also lead to devastating emotional and financial issues (Bushnik et al., 2012). The cost of reproductive treatments such as, in vitor fertilization, for infertile women can start anywhere from $4,700 per cycle, with women often needing more than one cycle to conceive (ARTUS Fertility Centre, 2013). Also, quality of life and health related quality of life scores are lower in infertile women compared to fertile women (Chachamovich et al., 2010). Therefore, due to the extreme physical, financial and emotional issues, effective treatments to decrease infertility are required.

There are numerous causes of infertility. Obesity, which is the increase in adipose (fat) tissue above a healthy amount for a given size (weight), has been under intense study as a key associated factor (Bushnik et al., 2012). Compared to normal weight women, obese women are three times more likely to experience infertility (Rich-Edwards et al., 1994). Causes of infertility in obese women are often related to changes in reproductive and metabolic hormones (Moran et al., 2011). As obesity incidences continue to rise among Canadian women, it is suggested that

infertility rates may also increase (Stats Canada, 2014a; Maclauso et al., 2010; Moran et al., 2011).

1.2 Obesity & Infertility

One method for categorizing an individual as obese is based on their body mass index (BMI, kg/m^2). BMI is calculated by dividing an individual's weight (kg) by their height (m) squared. A BMI between 18.0 kg/m^2 and 24.9 kg/m^2 is considered normal and an individual is considered overweight if their BMI is between 25.0 kg/m^2 and 29.9 kg/m^2. Obese status is given to those with a BMI greater or equal to 30.0 kg/m^2 and can be further separated into three different classes. Obesity class I is a BMI between 30.0kg/m^2 and 34.4 kg/m^2, obesity class II is a BMI between 35.0kg/m^2 and 39.0 kg/m^2, and obesity class III is a BMI greater or equal to 40.0kg/m^2 (CDC, 2013).

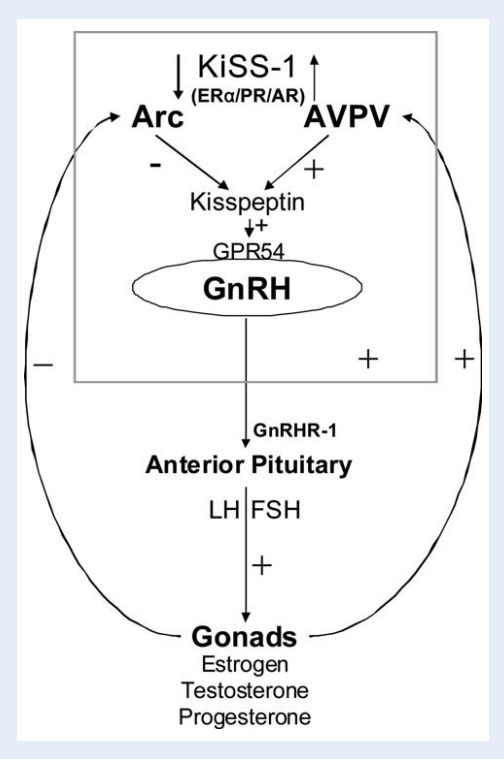
Obese women (class I, II & III) can experience abnormal changes in reproductive and metabolic hormones (Moran et al., 2011). Two important reproductive and metabolic hormones that are altered in an obese state are KiSS and leptin (Hausman, Barb, Lents, 2012; Oakley, Clifton, Steiner, 2009). In these obese states, circulating plasma KiSS levels decrease, whereas circulating plasma leptin levels increase (Quennell et al., 2011; Hausman et al., 2012). These hormone changes can affect other hormones, such as gonadotropin releasing hormone (GnRH), which is an essential hormone for reproductive function (Evans & Anderson, 2012).

1.3 Reproductive & Metabolic Hormones: Kisspeptin & Leptin

KiSS and leptin are involved in both reproductive and metabolic functions (Soulis & Kitraki, 2011; Fu & van den Pol, 2010; Evans & Anderson, 2012). Both of these hormones are involved in the hypothalamic-pituitary-gonadal (HPG) axis, which controls reproduction (Figure 1.1) (Roseweir & Millar, 2009). The HPG axis consists of the hypothalamus, pituitary and

gonads (ovaries). KiSS and leptin aid in the stimulation and secretion of GnRH from the arcuate nucleus (ARC), which is housed in the hypothalamus (Evans & Anderson, 2012) (Figure 1.2). Also leptin and KiSS both stimulate the satiety center of the body that is located within the ARC, ventromedial and paraventricular nuclei of the hypothalamus. This stimulation causes activation or inhibition of the production of specific satiety hormones and associated increases or decreases in eating behavior and energy expenditure (Soulis & Kitraki, 2011; Fu & van den Pol, 2010).

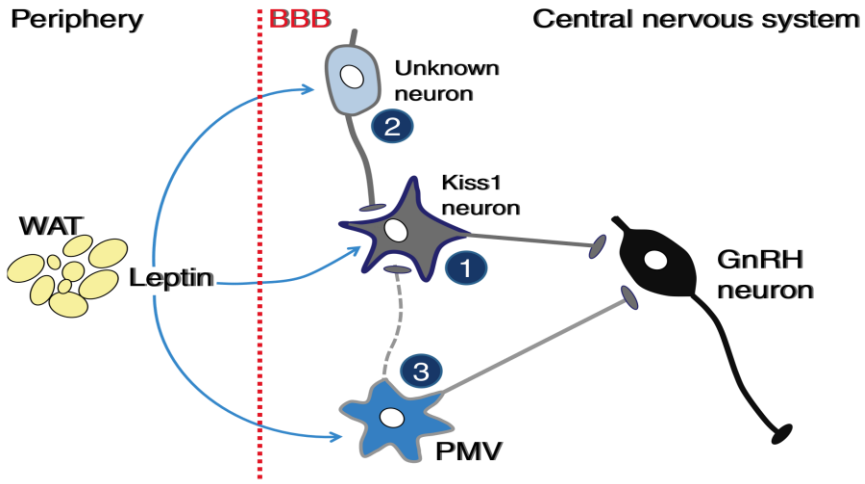
Figure 1.1 Hypothalamic-Pituitary-Gonadal Axis



(Roseweir & Millar, 2009)

Figure Description: The Hypothalamic-Pituitary-Gonadal (HPG) Axis contains the structures involved in the regulation of reproduction. The structures include the hypothalamus, which houses KiSS and GnRH neurons; the anterior pituitary, which contains luteinizing & follicle stimulating hormone and the ovaries contain the follicles

Figure 1.2 Interplay between leptin, KiSS and GnRH



(Tena-Sempere et al., 2012)

Figure Description: Circulating leptin concentrations signal to KiSS neurons, which in turn causes KiSS neurons to signal to GnRH neurons, causing increased reproductive function.

KiSS is a polypeptide hormone, which is produced by neurons in the ARC and is critical for reproduction (Oakley et al., 2009). KiSS is responsible for directly stimulating GnRH production and its release from the hypothalamus, which subsequently leads to the release of LH and FSH from the pituitary (Messager et al., 2002). LH and FHS then cause the maturation and release of ovarian follicles from the ovaries (Messager et al., 2002). KiSS' role in metabolism is not fully understood, however it has been shown to stimulate pro-opiomelanocortin (POMC) and inhibit neuropeptide Y (NPY), two important satiety and feeding hormones (Fu & van den Pol, 2010). A satiety hormone (POMC) is responsible for signaling a stop in energy intake and to increase energy expenditure where as a feeding hormone (NPY) is responsible for signaling an increase in energy intake and a decrease in energy expenditure (Soulis & Kitraki, 2011).

Leptin is produced in white adipose tissue. Its circulating levels are proportional to the amount of adipose tissue found within an individual (Soulis & Kitraki, 2011). As an anorexic hormone, leptin functions to decrease food intake and body weight by activating POMC and inhibiting NPY (Soulis & Kitraki, 2011). Without leptin, reproduction ceases. Individuals who

lack the leptin gene, express a mutation in it, or are leptin resistant present with infertility due to hypogonadism (Strobel, Issad, Camoin, Ozata, Strosberg, 1998; Farooqi et al., 1999; Tortoriello, McMinn, Chu, 2004). Several studies also show that leptin receptors (OR-Rb) are not found on GnRH neurons thereby suggesting a hormone between leptin and GnRH is needed for complete activation of the reproductive system (Quennell et al., 2009).

It is speculated that KiSS is a potential mediating hormone between leptin and the release of GnRH. This relationship between KiSS and leptin is supported by the presence of leptin receptors on KiSS producing neurons (Smith, Acohido, Clifton, Steiner. 2006). When exogenous leptin is injected into *Ob/Ob* (leptin deficient) food restricted or castrated mice, KiSS mRNA concentration increases (Luque, Kineman, Tena-Sempere, 2007; Smith et al., 2006). Experiments using cell lines, including mice hypothalamic cell lines (N6) and human GnRH secreting cell lines also demonstrate an increase in KiSS mRNA when leptin is injected (Luque et al., 2007; Morelli et al., 2008). However, abnormal KiSS mRNA concentrations and circulating leptin levels are found in obese participants or animals, suggesting there is a disruption in signaling between these two hormones in excess energy states (Tortoriello et al., 2004).

Obese women experience significantly higher circulating leptin levels compared to normal weight women. Matsubara, Maruka and Katayose (2002) found that circulating leptin levels in normal weight women were between 5.2 and 8.2 ng/ml. In contrast, obese women have significantly increased leptin levels (average 13.2±0.4 ng/ml) (Matsubara et al., 2002). The increase in circulating leptin levels in obese women is hypothesized to be due to a process called leptin resistance and may affect subsequent leptin cell signaling of reproductive hormones such as KiSS in a negative manor (Myers et al., 2012).

There is no specific definition of leptin resistance, however most literature refers to it as a) a decrease in the sensitivity of leptin to its receptors or b) the inability of leptin to be transported across the blood brain barrier (BBB) to its receptors in the ARC (Myers et al., 2012).

Overall, it is thought that leptin resistance impairs leptin's ability to signal to other hormones, such as those involved in reproduction. In 2011, Quennell et al. demonstrated a relationship between leptin resistance and decreased KiSS mRNA concentrations. Obese women rats (strain DBA/2J) demonstrated significantly elevated leptin levels (6.0ng/ml) compared to the chow fed controls (1ng/ml). Also compared to the controls, the obese rats had significantly decreased KiSS mRNA levels in the rostral periventricular region of the third ventricle in the brain (Qunennell et al., 2011). Although reproductive function was not measured in this study, in a similar experiment using leptin receptor deficient rats, it was found that there was no activation of KiSS or GnRH neurons in the ARC (Quennell et al., 2011). These results demonstrate how increased energy states and subsequent abnormal leptin levels in obese women may affect reproduction by disrupting leptin signaling and KiSS mRNA production (Quennell et al., 2011).

1.4 Exercise, Obesity & Infertility

Exercise is defined as a repetitive movement of the body that is planned and structured in order to maintain or increase health benefits (Niemen, 2011). Exercise is a very important treatment for weight loss in obese women and some studies have been associated with a decrease in circulating leptin levels (Nieman, 2011; Bouassida et al., 2010; Moran et al., 2011; Garber et al., 2011). Exercise that is accompanied by weight loss is also recommended for obese women who are having difficulty conceiving (American Society for Reproductive Medicine, 2008a; Moran et al., 2011). A review by Moran et al. (2011), showed obese women who lost 5 to 10% of body weight, which encompass fat and lean mass, had improved conception chances. Specifically, women experienced a significant reduction in the hormone dihydrotestosterone, which is a male sex hormone (Hollmann, Runnenaum & Gerhard, 1996) and significant increases in spontaneous ovulation (Clark et al., 1995; Clark, Thornley, Tomlinson, Galletly,

Norman, 1998). These results suggest exercise may therefore be an effective treatment for obese women trying to conceive due to its ability to decrease elevated leptin levels.

Literature is still inconclusive on whether or exercise causes significant decreases in leptin levels in obese women as studies such as Polak et al. (2006), Kondo Kobayshi, Murakami (2006), Sari, Balci, Balci, Karayalcin (2007) and Azizi (2012) all showed significant decreases in leptin in obese women after an exercise intervention. However studies including Kraemer et al. (1999), Volpe et al. (2008) and Arikawa, Thomas, Schmitz, Kurzer (2011) all failed to show significant decreases in leptin levels after an exercise intervention in obese women.

It is suggested that changes in energy, which is often measured as a decrease in fat mass (FM), percent body fat (%BF), weight or BMI, may be responsible for the decrease in elevated leptin levels in obese women involved in exercise interventions (Kraemer et al., 1999; Bouassida et al., 2010). When comparing the studies that showed a significant decrease in leptin in obese women after an exercise intervention to those that did not all studies that showed a decrease in leptin had a decrease in either FM, %BF and weight (Polak et al., 2006; Kondo et al., 2006) or BMI (Sari et al., 2007; Aziz, 2012). However, two out of the three studies that did not demonstrate a decrease in leptin levels also did not show a decrease in FM, %BF, weight or BMI (Kraemer et al., 1999; Volpe et al., 2008). Although Arikawa et al. (2011) did show a small but significant percent change in %BF (0.94%), they still failed to show a decrease in leptin levels. This may further suggest that a certain amount of adipose tissue may need to be decreased before leptin levels change (Kraemer et al., 1999).

Although there have been numerous studies conducted on weight loss and infertility, and weight loss and circulating leptin levels, there have been no studies that have examined changes in KiSS after an exercise intervention in obese women. As such, the relationship between weight loss, leptin, KiSS, reproduction and exercise remains unclear. It is therefore of interest to know if KiSS levels increase and elevated leptin levels decrease in parallel in obese women after an

exercise intervention. Understanding this relationship will allow for further studies to be conducted on the interplay between obesity, circulating KiSS and leptin concentrations.

1.5 Purpose

The purpose of this study was to determine the effects of a l2-week progressive aerobic exercise intervention on FM and %BF, as well as circulating leptin and KiSS levels in obese women.

1.6 Hypothesis

It was hypothesized that:

Obese women who were randomized into a 12-week progressive aerobic exercise intervention would show a decrease in FM, %BF and circulating leptin levels and an increase in circulating KiSS levels from baseline testing to mid-point testing and from mid-point testing to end-point testing. There would be no change in FM, %BF, circulating leptin or KiSS levels over the 12-week period in women randomized into the control group.

2.0 LITERATURE REVIEW

2.1 Infertility

Infertility is defined as the inability of a women to conceive within one year when the risk of conception is present (Bushnik et al., 2012). Currently, infertility affects between 11.5% and 15.7% of the Canadian women population (Bushnik et al., 2012). Unlike other medical issues, infertility is difficult to diagnose, as often the only outward sign is the failure of conception (Evers, 2002). Therefore, until a women actively attempts to conceive, she will not know if she is infertile or not. Thus, treating known risk factors of infertility is one of the most effective options to decrease its incidence.

Obesity, which is defined as an increase in adipose (fat) tissue above a healthy weight, is a risk factor and potential cause of infertility in women. Specifically, obese women are three times more likely to experience infertility compared to healthy weight women (Rich-Edwards et al., 1994). Obesity may impact fertility through abnormal production of hormones that regulate women reproduction (Moran et al., 2011). Given that the number of obese reproductive age women continues to rise in Canada, one might speculate that infertility may also be on the rise (Stats Canada, 2013a,b & 2014).

Consequences of infertility also include emotional, psychological and financial burdens. Infertile women have significantly decreased emotional, social and mental health scores (Souter, Hopton, Penney, Templeton, 2002). Their quality and health related quality of life scores, when compared to fertile women, are also significantly lower (Chachamovich et al., 2010). Women who experience infertility often turn to expensive reproductive treatments, such as in vitro fertilization (IVF), to increase conception chances. However, IVF and other reproductive treatments are extremely expensive, leaving these women and their families with increased debt loads. In Saskatchewan the cost of one cycle of IVF is $4,700, and the cost of medication associated with the procedure can range from $3,000 to $6,000 (ARTUS Fertility Centre, 2013).

Often there is a need for more than one round of IVF treatment; as well many provincial health care plans will only cover a portion of procedure, which causes even greater financial strain (Alberta Health Technologies Decision Process Assisted Reproduction Technologies, 2014). Along with the inability to conceive, the increased financial, emotional and psychological burden faced by infertile women, provides further incentive to treat risk factors, such as obesity (Souter et al., 2002).

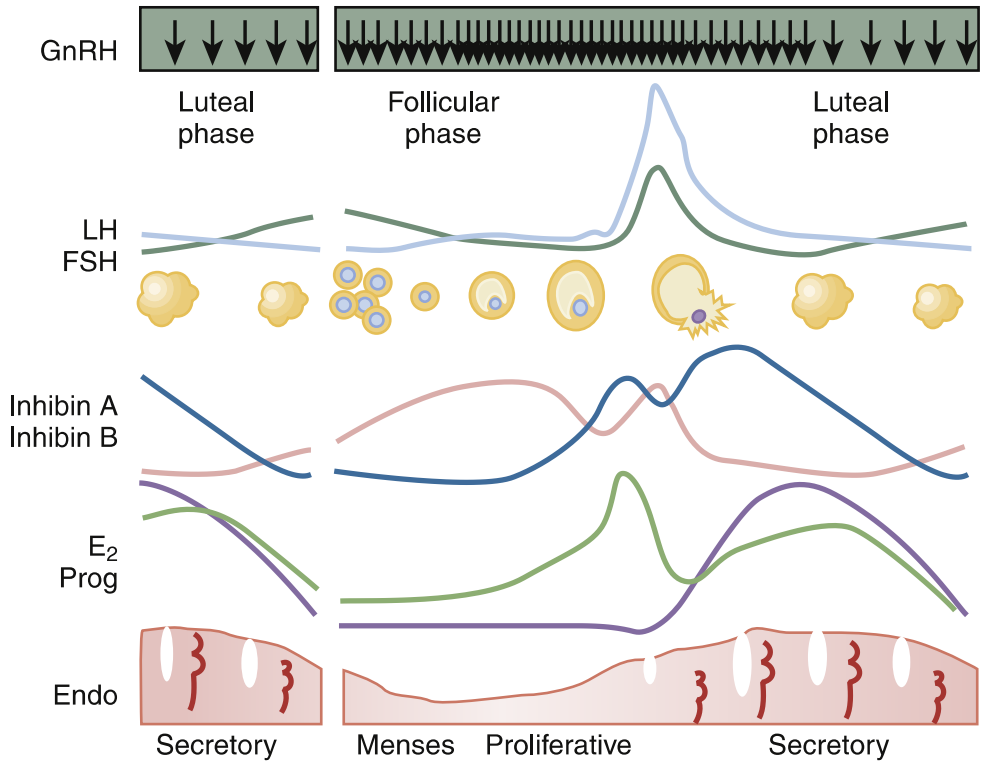
2.2 Female Reproduction

To understand the impact of obesity on reproduction it is important to understand the female reproductive cycle, otherwise known as the menstrual cycle, and the reproductive organs and hormones involved in its control.

2.2.1 The Menstrual Cycle

The function of the menstrual cycle is to cause the growth and development of a primordial follicle into a mature follicle (egg), as well as cause the release of a mature follicle from the ovaries. Once the egg is ovulated, it can then be fertilized and subsequently implanted in the uterus, leading to the eventual growth of a fetus (Hall, 2009). On average the menstrual cycle lasts 28-days and is split into two distinct phases, the follicular phase (FP) and the luteal phase (LP). Each phase lasts roughly 14-days (Hall, 2009). The FP is defined as the 'start' of the cycle (day 0) and is when menses (bleeding) begins. Menses lasts approximately the first 7-days of the FP (Hall, 2009). Ovulation of the mature follicle takes place after the FP (day 14) and is then followed by the LP (Figure 2.1) (Hall, 2009).

Figure 2.1 Reproductive Hormone Concentrations during the Menstrual Cycle



(Hall, 2009)

Figure Description: Change in reproductive hormone concentrations (GnRH, LH, FHS, Inhibin A, Inhibin B, E_2, Progesterone) and the endometrium over a regular menstrual cycle.

The growth of a primordial follicle to a mature follicle in preparation for ovulation happens during the FP. Follicle stimulating hormone (FSH) and luteinizing hormone (LH), which are both known as gonadotropins, are released from the anterior pituitary in the brain. Gonadotropins are hormones that regulate gonad (ovaries) function. FSH and LH cause the initial growth of six to twelve primordial follicles in the ovaries. However, only one primordial follicle will grow into a mature follicle for ovulation. Both FHS and LH increase during the FP and target different cells within the follicle (Figure 2.1). FHS targets its receptors on the granulosa cells and LH affects the thecal cell growth. As FSH and LH begin to rise, the maturing follicle secretes the women sex hormone, estradiol (E_2). E_2 suppresses any further rise in FHS and LH during the FP because it functions as a negative feedback system (Figure 2.1). It is

thought that E_2 most likely relays its negative feedback on FSH and LH via the hypothalamus since GnRH amplitude is significantly decreased in the presence of E_2 (Hall, 2009). GnRH causes the production and secretion of LH and FSH from the anterior pituitary. E_2 may also act directly on FSH and LH neurons in the anterior pituitary, however this remains inconclusive (Hall, 2009). FSH production and secretion is further suppressed by the release of the hormones inhibin A & B from the granulosa cells in the maturing follicle (Stenvers & Findlay, 2010).

Late in the FP, E_2 interaction with LH and FSH changes from a negative to a positive feedback system. Surging E_2 levels from the maturing follicle at the end of the FP are responsible for the switch of E_2 from the negative to positive feedback trigger (Hall, 2009). Specifically, the increase in circulating E_2 levels stimulates LH concentrations to peak. It is the rapid increase and peak in LH that causes ovulation of the mature follicle to occur (Figure 2.1) (Hall, 2009).

The LP starts after ovulation has taken place. It is characterized by the decrease in FSH and LH and the degradation of the mature follicle if fertilization and implantation has not occurred (Hall, 2009). The ovulated unfertilized follicle, known as the corpus luteum, continues to produce and secrete E_2 and inhibins. Also, the corpus luteum begins producing and secreting progesterone. E_2 and progesterone cause the decrease in FSH and LH release via negative feedback (Hall, 2009). Eventually, the corpus luteum degrades, triggering E_2, inhibins and progesterone levels to fall. The decrease of these hormones removes the inhibition on FSH and LH, allowing their levels to rise. This increase of FSH and LH signals the start of a new menstrual cycle (Hall, 2009) (Figure 2.1).

In general a women' first menses (menarche) occurs at the end of puberty, which roughly corresponds to a chronological age of 12.9 in Canadian girls (Mihm, Gangooly, Muttukrishna, 2011; Malina, Bouchard, Bar-Or, 2004). It is hypothesized that up until the beginning of puberty FSH and LH levels are kept at very low levels by the hormone gonadotropin-inhibiting hormone

(GnIR). GnIR is produced by the hypothalamus and potentially inhibits GnRH as well as LH and FSH production and secretion (Ubuka, Son, Bentley, Millar, Tsutsui, 2013). At the onset of puberty, which is around age 8 or 9 in women, changes in GnIR and GnRH levels take place, GnIR decreases and GnRH increases. As GnRH release increases the magnitude in the release of FSH and LH, E_2 increases, eventually leading to menarche (Mihm et al., 2011; Ubuka et al., 2013).

The window of reproduction in women is finite. As women age, they begin to experience irregularities in their menstrual cycle. These irregularities are known as the onset of menopause (Mihm et al., 2011). Menopause is the depletion of a women's ovarian (follicle) reserve, which causes changes in, and the eventual stoppage of reproductive hormones, including E_2, progesterone and inhibin concentrations (Lobo, 2009). On average, women enter menopause at around age 47 (Mihm et al., 2011). When the reproductive cycle ceases this signals the end of the reproductive years (Lobo, 2009).

2.2.2 Control of Menstrual Cycle

GnRH is the master hormone regulating the menstrual cycle, as it controls production and secretion of LH and FHS. Recently scientists discovered that kisspeptin (KiSS) is the hormone that controls and stimulates GnRH (Messager et al., 2005). KiSS is a neuropeptide produced in the hypothalamus (Messager et al., 2005; Oakley et al., 2009).

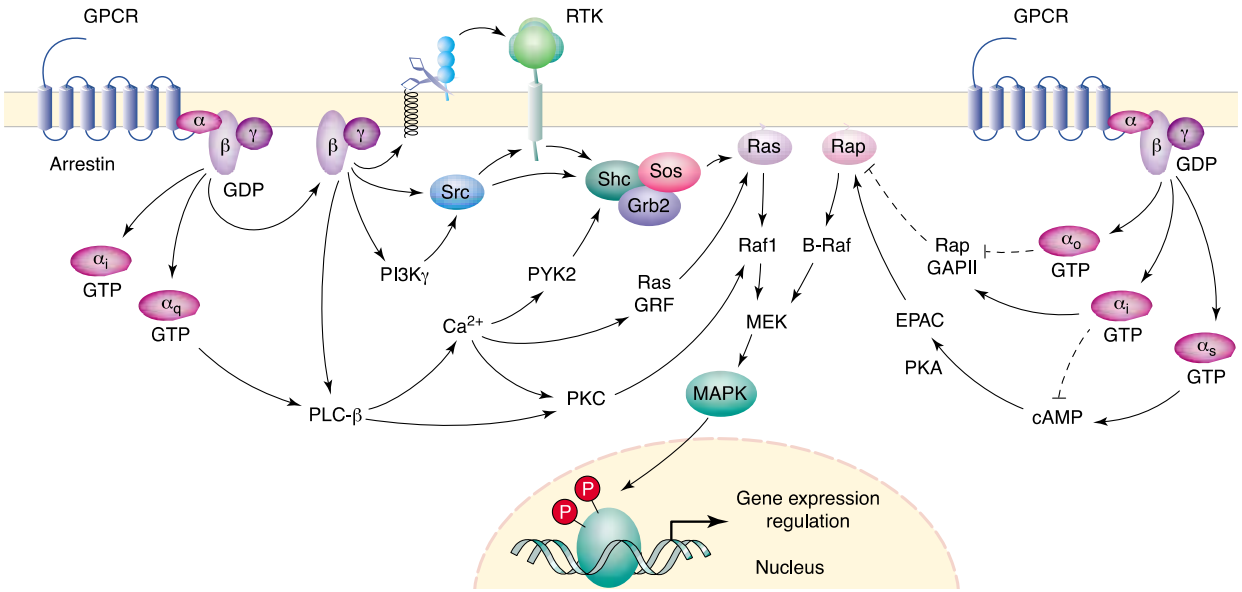
2.2.2.1 Gonadotropin Releasing Hormone (GnRH)

GnRH is a neuropeptide hormone produced and secreted in a pulsating fashion from neurons in the ARC of the hypothalamus (Hall, 2009). Once released, GnRH travels via the hypophyseal portal to the anterior pituitary where it stimulates the production and secretion of LH and FSH (Clifton & Steiner, 2009; Bliss, Navratil, Xia, Roberson, 2010). There are two

different GnRH isoforms, GnRH I or GnRH II, found in humans, however GnRH I (subsequently referred to as GnRH) is responsible for controlling the menstrual cycle (Clifton & Steiner, 2009). Specifically, high pulse secretion frequencies of GnRH cause LH secretion, whereas low pulse secretion frequencies cause FSH secretion. It is hypothesized that the high frequency GnRH pulses cause the drastic rise in circulating LH concentrations immediately before ovulation in the LP (Bliss et al., 2010).

When GnRH binds to GnRH receptors (GnRH-R), which are G-protein coupled receptors (GPCR), found on LH and FSH neurons in the anterior pituitary, LH and FSH secretion increases (Bliss et al., 2010). A GPCR consists of seven connected trans-membrane domains; with the ligand (hormone) binding site found on the extracellular membrane (Figure 2.2) (Naor, 2009; Rosenbaum, Rasmussen, Kobilka, 2009). The overall structure of the GnRH-R end on the intracellular surface and is responsible for the activation of cell signaling cascades, resulting in the production or secretion of LH or FSH (Rosenbaum et al., 2009). Specifically, the binding of GnRH to its GnRH-R activates the GPCR-subunits (α, β, γ) and causes the exchange of energy from one nucleotide, guanosine diphosphate (GDP), for another, guaosine-5'-triphosphate (GTP) (Naor, 2009). Nucleotides are specific cell energy carriers. The transfer of a phosphate to GDP causes the disassociation of GPCR-α from the GPCR β,γ subunits; leading to activation of signaling proteins further down the cascade (Figure 2.2) (Naor, 2009). The activation of the Mitogen Activated Protein Kinase (MAPK) signaling cascade by GnRH-R is responsible for the production and secretion of LH and FSH (Naor, 2009).

Figure 2.2 Cell Signaling Pathways of GnRH G-protein Coupled Receptors (GPCR)



(Marinissen & Gutkind, 2001)

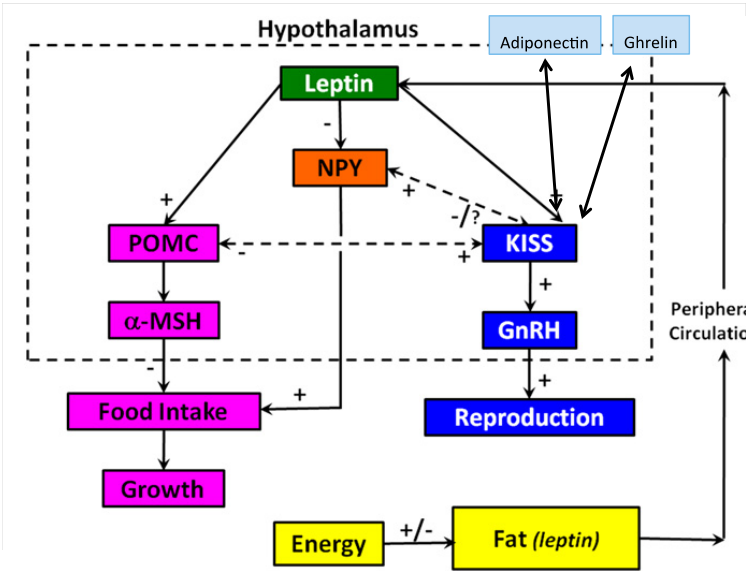
Figure Description: Activation of the cell-signaling pathway by GnRH and GnRH-R triggers the MAPK signaling cascade. The MAPK cascade causes increases in the transcription of other hormones in the nucleus of the cell, including FSH and LH. The GPCR subunits (α, β, γ) are activated by the binding of a ligand (GnRH) to the extracellular domain of GPCR. Once activated GPCR α subunit dissociates from the β, γ subunits causing the eventual transcription of other hormones.

2.2.2.2 Kisspeptin (KiSS)

KiSS, a neuropeptide hormone produced and released from neurons in the ARC, in humans and animals, controls GnRH secretion (Messager et al., 2005). KiSS is produced by the KiSS-1 gene, which was first found to be a tumor-suppressing hormone called metastin (Lee et al., 1996). However, KiSS was later deemed an essential hormone for reproduction as mutations in KiSS or its receptor (KiSS-R) caused hypogonadism (lack of sex hormone production) and infertility in humans (de Roux et al., 2003; Seminara et al., 2003). KiSS is also involved in metabolic functions as numerous studies have demonstrated reciprocal activation between KiSS and the metabolic hormones leptin, adiponectin, neuropeptide Y (NPY), ghrelin and proopiomelacortin (POMC) (Figure 2.3). (Fu & van den Pol, 2010). However, there are still

many gaps surrounding the intricate function of KiSS in reproductive and metabolic processes, indicating further research is needed.

Figure 2.3 Interplay Between KiSS, Reproductive & Metabolic Hormones



(Modified from Hausman et al., 2012)

Figure Description: Increases in circulating leptin concentrations, signals increases in KISS messenger ribonucleic acid (mRNA) and POMC regulation and a decrease in NPY regulation. Increases in KiSS leads to increased reproductive function. Reciprocal inhibition between KiSS and the hormones POMC, NPY, adiponectin and ghrelin is also present.

Since the original discovery of KiSS more evidence has accumulated to support its role in reproduction. There are five key pieces of evidence that suggest KiSS is necessary for reproduction. (i) First the KiSS-R, which is a GPCR, is localized in the ARC of the hypothalamus. This provides a conducive environment for the activation of GnRH neurons, because GnRH neurons are also housed in the ARC (Messager et al., 2005). (ii) Second, KiSS stimulates the secretion of GnRH possibly through the activation of the $G\alpha_{q/11}$- pathway (Muir et al., 2001). (iii) Third, exogenous KiSS injections are associated with significant increases in GnRH secretion (Messager et al, 2005; Plant, Ramaswamy, DiPietro, 2006; Han et al., 2005; Shabab et al., 2005; Matsui, Takatsu, Kumano, Matsumoto, Ohtaki, 2004). This increase in

GnRH secretion has been specifically shown in animal models such as ewe, monkey, mice and rat models (Messager et al, 2005; Plant et al., 2006; Han et al., 2006; Shabab et al., 2005; Matsui et al., 2004). (iv) Fourth, a lack in KiSS-R is associated with a lack of LH and FSH surges compared to controls (Messager et al., 2005). Also lack of KiSS neurons in the ARC of women mice are associated with a significant decrease in complete oestrous cycles and LH pulse frequency compared to controls (Beale et al., 2014). (v) Finally, exogenous injections of KiSS restore FHS and LH secretion in KiSS deficient rodent models (Navarro et al., 2005). As the evidence suggests, KiSS is highly involved in reproduction, thus the continuing study of KiSS is crucial to determine its precise functions in both reproduction and metabolism.

Production of KiSS, like GnRH, occurs in the ARC as well as in the anteroventral periventricular nucleus of the hypothalamus (Clarkson & Herbison, 2009). Sagittal brain slices have only been studied in humans, making it difficult to identify KiSS neurons in the rostral periventricular of the third ventricle and the medial preoptic area as has been observed in rodents and ewes (Rometo, Krajewski, Voytko, Rance, 2007; Oakley et al., 2009). Other areas of the human body that contain small amounts of KiSS neurons include the placenta, pancreas, liver and small intestine (Oakley, et al., 2009).

KiSS is produced as a 154-amino acid pro-hormone that can be proteolytically cleaved by pro-hormone convertase into a 54-amino acid protein known as KiSS-54 (Oakley et al., 2009). KiSS-54 can again be cleaved into smaller amino acid proteins called kisspeptins (KiSS-10, KiSS-13, KiSS-14) (Oakley et al., 2010). All four variations of KiSS are biologically active and bind to KiSS-R, however the relevance of the different forms of KiSS remains unclear (Kotani et al., 2001).

Research has demonstrated that E_2 and the metabolic hormone leptin control KiSS production (Franceschini et al., 2006; Gottsch et al., 2009; Quennell et al., 2011). It is still inconclusive however, if other reproductive (Neurokinin B, Dynorphin) and metabolic hormones

(NPY, POMC) also contribute to KiSS production (Figure 2.3) (Lehman, Coolen, Goodman, 2010; Backholer et al., 2010).

Control of KiSS mRNA production by E_2 is completed through a negative or positive feedback system depending on where the KiSS producing neurons are located. KiSS neurons in the ARC are under a negative E_2 feedback system. Increases in E_2 are associated with significant decreases in KiSS mRNA expression in the ARC (Smith et al., 2005). However, increases in E_2 are associated with increases in KiSS mRNA in the anteroventral periventricular nucleus (Smith et al., 2005; Gottsch et al., 2009).

Estradiol alpha-receptors ($E_2\alpha$) are present on KiSS neurons in ARC of sheep and mice (Franceschini et al., 2006; Smith et al., 2005). This provides a potential environment for reproductive signaling to take place as GnRH neurons that contain KiSS-R are present in the ARC as well. Additionally, rodents and primates who have their ovaries surgically removed (OVX) have significantly increased KiSS mRNA expression in the ARC compared to non-OVX control (Smith et al., 2005; Rometo et al., 2007; Gottsch et al., 2009). It is hypothesized that the removal of the ovaries, which contain the E_2 producing follicles, leads to significantly reduced E_2 levels. To further support this observation, when OVX mice or monkeys had E_2 replacement capsules surgically implanted, KiSS mRNA levels in the ARC significantly decreased (Smith et al., 2005; Rometo et al., 2007). Also, mice that lacked $E_2\alpha$ and were injected with E_2 did not display significantly decreased KiSS mRNA levels, whereas mice with $E_2\alpha$ did experience a significant decrease in KiSS mRNA when injected with E_2 (Gottsch et al., 2009).

Although the relationship between E_2 and KiSS in humans is still not fully understood, it has been shown that women who have finished menopause have an increased number of KiSS producing neurons (Rotmeto et al., 2007). These neurons are also increased in size (Rotmeto et al., 2007). In contrast, Chan, Butler, Sidhourn, Pinnell, Seminara (2012) demonstrated in a cross-sectional study that changes in KiSS mRNA in relation to the fluctuating E_2 levels was not

significantly different between the FP and LP of the menstrual cycle in women. One would postulate that KiSS mRNA levels would change as E_2 levels change throughout the menstrual cycle however, these results suggest otherwise. It is important to note however that this study had a very small sample size (27) and an uneven number of women in each group (10 FP, 3 pre-ovulatory, 14 LP), which may have potentially skewed the results (Chan et al., 2012). The regulation of KiSS mRNA by E_2 is well supported in the animal literature however further investigations focusing on humans are imperative.

The metabolic hormone leptin may also regulate KiSS production in the ARC. Results from various studies suggest that increases in circulating leptin concentrations are followed by increases in KiSS production (Backholer et al., 2010). The specific relationship between leptin and KiSS however is still under investigation as there are a number of other metabolic hormones that leptin controls that may also regulate KiSS production (figure 2.3) (Luque et al., 2007; Backholer et al., 2010). To complicate matters further, abnormally high leptin levels, as seen in obese populations, may cause the down regulation of KiSS production due to leptin resistance (Myer et al., 2012; Quennell et al., 2011).

2.3 Metabolism & Reproduction

2.3.1 Leptin

Hormones involved in controlling the metabolic state of the body also exert control over reproductive function (Evans & Anderson, 2012). Leptin is an anorexigenic hormone produced by adipose tissue. The metabolic function of an anorexigenic hormone is to decrease energy intake and increase energy expenditure. In the specific case of leptin, increased energy intake causes an increase in circulating leptin levels. This increase in leptin eventually triggers a decrease in energy intake and an increase in energy expenditure (Perboni, Ueno, Mantovani, Inui, 2009). Leptin is also implicated in reproduction (Hausmen et al., 2012). Individuals and/or

animals who possess a mutation in their leptin gene, are leptin receptor deficient or are 'leptin resistant' present with reduced levels of fertility or are classified as infertile (Strobel al et., 1998; Clement et al., 1998; Tortoriello et al., 2004). The recent association of leptin and increases in KiSS mRNA (Luque et al., 2007; Smith, et al., 2006) highlight the possibility of a more prominent role of leptin in reproduction

2.3.1.1 Leptin Production

In general, circulating leptin concentrations are proportional to the amount of adipose tissue present (Lee & Fried, 2009). Insulin, glucocorticoids (GC), cytokines and short-term changes in nutrient status can also alter acute and chronic levels of circulating leptin (Lee & Fried, 2009; Lynch et al., 2006).

Insulin controls both the mRNA concentration and the secretion of leptin (Lee & Fried, 2009). The majority of studies indicate that exposure to insulin increases leptin mRNA expression over a 24 – 48 hour period (Moreno-Aliage, Stanhope, Havel, 2001; Lee, Yang, Gong, Fried, 2007; Lee & Fried, 2009). This increase is mediated via the phosphoinositide 3-kinase (PI3K) and/or the mammalian target of rapamycin (mTOR) cell signaling pathways (Lee & Fried, 2009). Insulin also increases post-translational secretion of leptin. In both rat and human tissue, leptin secretion from adipocytes increased 120-minutes after exposure to insulin (Bradley & Cheatham, 1999). Interestingly, in fasted states insulin does not display the same control over leptin production or secretion; therefore the effect of insulin on leptin concentrations may be dependent of the energy state of the body (Fried & Lee, 2009).

GC and cytokines may also control long-term leptin mRNA expression (Lee & Fried, 2009). When given oral GC or dexamenthasone (synthetic GC) leptin mRNA levels in human subjects doubled within a 24 hours (Larsson & Ahren, 1996; Papaspyrou-Rao, Schneider, Petersen, Fried, 1997) to 96 hour period (Newcomer et al., 1998). Moreover, when GC were

given in conjunction with insulin they acted synergistically to significantly increase leptin mRNA concentrations (Bradley & Cheatham, 1999). Independent of GC, the cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) and the neuropeptide melanin-concentrating hormone (MCH) also significantly increased leptin mRNA (Lee & fried, 2009). When paired with CG or dexamenthasone, MCH and TNFα increased leptin expression respectively (Trujillo et al., 2004; Trujillo et al., 2006). However when not administered with GC, TNFα was associated with a significant decrease in leptin expression (Trujillo et al., 2006). Obese individuals present with increased levels of GC, TNFα and IL-6, highlighting the impact obesity has on numerous other hormones that can affect the long-term expression of leptin (Finck & Johnson, 2002).

Short-term expression of leptin mRNA is regulated by nutritional signals. Free fatty acids (FFA) decrease leptin mRNA. It has been suggested that this decrease is due to the antagonist effect of FFA on insulin signaling (Lee & Fried, 2009). In addition, nutritional signals may also regulate short-term leptin secretion. Similar to leptin mRNA concentrations FFA also reduce leptin secretion from adipose tissue (Lee & Fried, 2009). However, postprandial rises in branch chain amino acids, specifically leucine, cause a significant increase in circulating leptin (Lynch et al., 2006).

Independent of insulin, glucose precipitates an increase in leptin mRNA, possibly through the hexosamine biosynthesis pathway (HBP) (Lee & Fried, 2009). Three percent of glucose that enters an adipose cell is shuttled into the HBP via its conversion to glucosamine-6-phosphate by the enzymes hexokinase, phosphoglucose isomerase and glutamine fructose-6-phospahte aminotransferase (Shirato, Kizaki, Ohno, Imaizumi, 2012). Glucosamine-6-phosphate is phosphorylated numerous times to produce uridine-5’-diphosphate-N-acetyglucaoslation (UDP-GlcNAc). UDP-GlcNAc increases the signal transduction of leptin production and thus increases leptin mRNA concentrations (Shirato et al., 2012). However, the increase in leptin

concentration may also be due to the increase in adenosine triphosphate (ATP) from glucose entering the glycolytic pathway instead the HBP (Lee & Fried, 2009). Thus, increases in circulating leptin levels may be due to the general increase in metabolic energy from glycolysis, signaled by elevated ATP levels or by the production of UP5' and the HBP (Lee & Fried, 2009; Shirato et al., 2012).

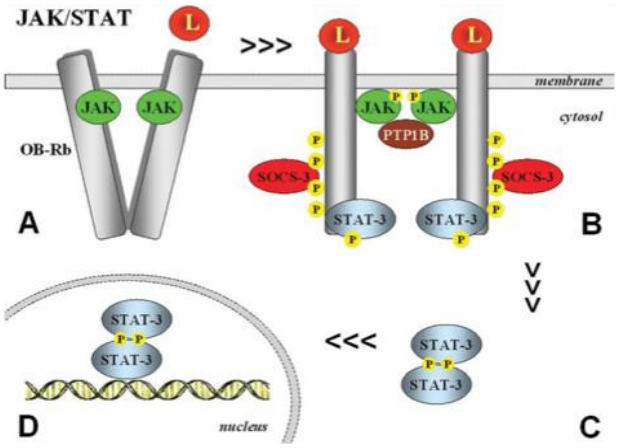
2.3.1.2 Leptin Signaling

Once leptin is produced and secreted from adipose tissue it travels via the circulation until it binds to one of its receptors. Leptin receptors are trans-membrane cytokine receptors that span both the inner and outer membrane of the cell (Perboni et al., 2009; Wang, Lupardus, LaPorte, Garcia, 2009). The ligand binds to the extracellular part of the receptor, causing the activation of signaling cascades and the production of metabolic and reproductive hormones (Wang et al., 2009). Leptin receptors are found throughout the body including muscle and liver tissue; however the most abundant amount of leptin receptors, as similarly noted for KiSS-R, are found in the ARC of the hypothalamus (Fruhbeck, 2006). Once leptin enters the brain it binds to one of its six different receptor isoforms. The six isoforms (OB-Ra, OB-Rb, OB-Rc, OB-Rd, OB-Re and OB-Rf) are categorized as short, long or secreted receptors (Cottrell & Mercer, 2012). The long form receptors (OB-Rb) are used in signal transduction whereas the short form receptors (OB-Ra, OB-Rc, OB-Rd, OB-Rf) are only responsible for the transport of leptin across the blood brain barrier (BBB) (Fruhbeck, 2006; Cottrell & Mercer, 2012). The secreted or soluble leptin receptor (OB-Re) that is found in circulation binds to leptin, and decreases its bioavailability (Fruhbeck, 2006).

When leptin binds to OB-Rb it activates a cascade of cell signaling proteins. Activation of these cascades leads to increased production of specific metabolic and reproductive hormones. The major signaling pathway of OB-Rb is the Janus Kinase/Signal Transducer 3 (JAK/STAT3)

(Figure 2.4) (Fruhbeck, 2006). Once activated, the JAK/STAT3 signaling cascade causes the phosphorylation of STAT3 within the cell cytosol. STAT3 then travels from the cytosol to the inside of the nucleus to stimulate the transcription of new hormones (Fruhbeck, 2006). Although the majority of signaling is done via the JAK/STAT3 pathway, OB-Rb can activate other pathways including MAPK, Phosphoinositide 3-kinase (PI3K)/Phosphodiesterase 3B (PDE3B)/Cyclic Adenosine Monophosphate (cAMP) and 5' Adenosine Monophosphate-Activated Protein Kinase (AMPK) (Fruhbeck, 2006). Both JAK/STAT3 and other signaling cascades are implicated in the increase and decrease of specific metabolic and reproductive hormones.

Figure 2.4 OB-Rb & Janus Kinase/Signal Transducer 3 (JAK/STAT) Signaling Pathway



(Fruhbeck, 2006)

Figure Description: The binding of leptin to OB-Rb causes the phosphorylation of STAT3. STAT3 then disassociates from OB-Rb and travel to the nucleus of the cell where it initiates transcription of other hormones.

The ability of leptin to properly activate signaling cascades responsible for metabolic and reproductive function may be compromised by obesity. Increased nutrients, such as FFA observed in obese states, negatively impact leptin activated signaling cascades. Consequently,

leptin mediated metabolic and reproductive functions are affected (Myers et al., 2012). Obese states are also accompanied by a significantly increased circulating leptin concentrations (Myers et al., 2012). Elevated leptin concentrations in obese states seem counter intuitive as leptin levels normally decrease in environments with increased nutrient and/or stored energy sources. However the phenomenon of elevated leptin levels and altered metabolic and reproductive function may be a specific consequence of obese states known as leptin resistance (Myers et al., 2012).

2.3.1.3 Leptin Resistance

Obese women have significantly elevated circulating leptin concentration compared to normal weight women and it is suggested that this is due to the inability of leptin to signal other hormones (Myers et al., 2012). Circulating leptin levels for normal weight women are between 5.2 – 8.1 ng/ml, however obese women have an average circulating leptin levels of 13.2 ng/ml (Matsubara et al., 2002). This significant increase in leptin has been associated with abnormal menstrual cycles and infertility (Sahu, 2004; Evans & Anderson, 2012). In 2004, Tortoriello et al., found that pregnancy rates of diet induced, leptin resistant mice (strain DBA/2J) were 60% less compared to the normal weight control group. While the rise in leptin seems contradictory to what is "normally" observed in response to fed and fasted states, it is believed to ensue due to leptin resistance.

Currently there is no concrete definition of leptin resistance, however most literature describes it as a decrease in the sensitivity of leptin to its OB-Rb, or the inability of leptin to reach its OB-Rb (Myers et al., 2012). Consequently, functions that leptin normally signals will not happen (Myers et al., 2012). Also, due to the inhibition of leptin signaling, leptin production and/or secretion continues, regardless of the energy level of the body. Ultimately, this means that

leptin will continue to be produced, even in high energy states, leading to significantly increased circulating levels (Myers et al., 2012).

There are numerous ways in which leptin signaling may be impaired by leptin resistance. First, leptin transportation across the BBB may be disrupted. Short-form leptin receptors on the external surface of the BBB transport leptin from the circulation to its OB-Rb in the ARC (Cottrell & Mercer, 2012). However in obese states, hypertriglyceridemia (spillover of triglycerides [TG] from adipose tissue into circulation) prevents leptin from binding to its short form receptors. TG bind themselves to the short form receptors, inhibiting leptin from reaching its OB-Rb and preventing signaling (Cottrell & Mercer, 2012).

Second, negative feedback by suppressor cytokine signaling 3 (SCOS3) may impair leptin signaling. SCOS3 is a signaling protein whose circulating levels increase as leptin levels increase. SCOS3 binds to OB-Rb in the hypothalamus causing inactivation of leptin's main signaling pathway JAK/STAT3, thus preventing signaling of subsequent metabolic or reproductive hormones (Bjorbaek, El-Hachimi, Frantz, Flier, 1999; Mori et al., 2004; Fukuda, Williams, Gaustron, Elmquist, 2011).

Third, faulty leptin protein folding by 'stressed' endoplasmic reticulum (ER) can affect leptin signaling. The ER is a cell organelle, which is essential for protein production, including leptin. If the ER is damaged or stressed it causes miss-folded proteins, otherwise known as the Unfolded Protein Response (UPR) (Xu, Baily-Maitre, Reed, 2005). In obese states, the ER is subjected to high amounts of circulating TG that increase ER stress and attenuate UPR (Xu et al., 2005). Damaged proteins, due to the UPR, are unfit for their function, including signaling increases in other hormones (Xu et al., 2005). Obese mice with leptin resistance have presented with the UPR (Ozcan et al., 2009). Further research on animals has shown that the UPR and leptin resistance affects leptin's signaling of KiSS. Diet induced obese women rodents (strain DBA/2J) displayed significantly increased leptin levels and significantly decreased KiSS gene

expression in the ARC compared to the controls (Quennell et al., 2011). Since, in a leptin resistant state, leptin does not signal properly, rats with leptin deficiency may provide a suitable model for further studying leptin resistance.

It is hypothesized the changes to leptin's potential to signal other reproductive and metabolic hormones, may contribute to in infertility and abnormal metabolism (Myers et al., 2012). Therefore it is imperative to research targeted interventions to decrease excess energy and/or nutrient levels in obese states; as excess energy and/or nutrient levels are responsible for elevated leptin levels and impaired leptin signaling.

2.3.1.4 Metabolic Function

Leptin's ability to sense short and long term energy reserves of the body is an extremely important function, as processes involving energy regulation, such as metabolism, would not otherwise be able to take place. Without the appropriate amount of energy, such as displayed in leptin resistant states, women may experience changes in subsequent metabolic hormones (Evans & Anderson, 2012; Moran et al., 2011).

Leptin's acute circulatory concentrations are influenced by changes in nutrient status, thus allowing leptin levels to dictate short-term energy regulation (decrease energy intake and increase energy expenditure). Postprandial increases in blood glucose or the amino acid leucine, cause the production and/or release of leptin from adipose tissue (Soulis & Kitraki, 2011). Once released, leptin binds to OB-Rb on neurons in the ARC, where the JAK/STAT3 pathway is activated, causing the release of other anorexigenic neuropeptide hormones, including POMC and α-melanocyte-stimulating hormone (α-MSH) (Figure 2.3) (Soulis & Kitraki, 2011). POMC and α-MSH decrease appetite and increase energy expenditure (Soulis & Kitraki, 2011). It is suggested that signaling pathways independent of JAK/STAT3 are activated by leptin receptors, inhibiting production of the orexigenic neuropeptide hormones, NPY and agoui-related peptide

(ARP) (Soulis & Kitraki, 2011). Orexigenic hormone, including NPY and ARP increase appetite and decrease energy expenditure (Soulis & Kitraki, 2011). When energy levels decrease, which causes circulating leptin levels to decrease, the inhibition of NPY and ARP by leptin is removed and inhibition of POMC and α-MSH occurs (Soulis & Kitraki, 2011).

Similar to its ability to regulate short-term energy availability leptin also regulates long-term energy availability. This is done through its regulation of lipid metabolism (Margetik, Gazzola, Pegg, 2002; Ceddia, 2005). Like its short-term function, leptin's long-term function is to decrease energy stores and increase energy expenditure. This is caused by preventing the storage of TG in adipose tissue while increasing the breakdown of TG already in storage. Activation of OB-Rb prevents the conversion and storage of FFA to TG by activating via phosphorylation the AMPK signaling pathway. Activation of AMPK inhibits acetyl-Coenzyme A Carboxylase (ACC) by phosphorylating its β subunit (Minokoshi et al., 2002; Ceddia, 2005). ACC is an enzyme responsible for the storage of TG through the conversion of acetyl-Co A to malonyl-CoA (Minokoshi et al., 2002; Ceddia, 2005). Triglyceride storage is further prevented by decreasing the amount of fatty acid transporters, including fatty acid translocase and fatty plasma membrane fatty acid binding proteins, both of which are found on the outside of adipose cell membranes (Ceddia, 2005).

Leptin increases energy expenditure by breaking down previously stored fat in two predominate ways. Firstly, leptin increases the activity of the enzyme hormone sensitive lipase (HSL). HSL is one of three enzymes responsible for the break down of stored TG into FFA and glycerol (Kraemer & Shen, 2003). Second, the AMPK signaling pathway that is activated by leptin, breaks down TG. TG are broken down by increasing beta-oxidation in adipose, muscle and liver tissue via increases the enzyme carnitine palmitoyltransferase-1 (Ceddia, 2005). Beta-oxidation is the process used to breakdown FFA, allowing them to enter the electron transport chain and produce ATP for energy needs.

However, in leptin resistant states, leptin signaling is compromised causing altered metabolic hormones that continue to exacerbate increased nutrient and obese states. Specifically, NPY is found to be significantly elevated in leptin resistant states (Tortoriello et al., 2004). Recall, NPY signals increases in energy intake and a decreases in energy expenditure; therefore there continues to be an excess of nutrients in the body leading to increased leptin levels and continual impairment of leptin signaling (Soulis & Kitraki, 2011). The anorexigenic hormone desacetyl-α-MSH is also compromised in obese states. Desacetyl-α-MSH levels decrease, which prevents the decrease in energy intake and increase in energy expenditure (Enriori et al., 2007). The consequence of increased nutrient and energy not only affect the signaling of other metabolic hormones via leptin, but also intensifies the conditions that lead to increased energy and leptin resistance states. These drastic alterations to energy regulation will eventually affect other body functions that are controlled by energy regulation, such as reproduction.

2.3.1.5 Reproductive Function

The Energy Availability Hypothesis states a certain level of energy is needed for basic functions, such as brain function, to take place (Loucks, 2003). If energy demands for basic functions are not met, systems not immediate for survival, including reproduction, will cease to effectively function (Loucks, 2003). Therefore, hormones that can sense and regulate energy intake and expenditure are extremely important to ensure a properly functioning reproductive system (Loucks 2003). It is suggested that leptin is one such hormone that regulates energy availability for reproductive function.

Low energy states are accompanied by reduced or otherwise abnormal reproductive function, including reduced LH concentrations (Loucks, Verdun, Heath, 1998). This suggests GnRH levels may be impaired by low energy states, since GnRH is responsible for LH production and release. Interestingly however, GnRH neurons do not contain receptors that sense

energy availability. This indicates that another hormone, potentially leptin, may play a role in establishing circulating GnRH levels and reproduction. To support the link between energy availability such as, leptin and circulating levels of reproductive hormones, Welt et al. (2004) demonstrated that women who had a low BMI due to increased energy expenditure were amenorrheic (stoppage of menstrual cycle) and were leptin deficient. When recombinant leptin injections were given to 8 of these 14 women, significant increases in reproductive function including, increases in maximal follicular diameter, number of dominant follicles and ovarian volume were seen (Welt et al., 2004). Evidence such as this indicates that energy availability and leptin are important components of reproductive function.

As demonstrated, low energy states are accompanied by abnormal reproductive function, however high-energy states, such as obesity, are also followed by negative changes in reproduction. Tortoriello et al., (2004) demonstrated that diet induced obese mice experienced over 60% less pregnancies compared to normal weight controls. However, what Tortoriello et al., 2004 also found was that these diet induced obese, leptin resistance mice also had 50 – 95% less hypothalamic GnRH transcription compared to the controls. This study demonstrates the negative effect excess energy has on leptin levels and reproductive function.

Originally, it was hypothesized that leptin directly signaled GnRH production, however more recent studies indicate that GnRH neurons do not contain the short or long form leptin receptor (Hausman et al., 2012). As well, Quennell et al. (2009) found that leptin's main signaling pathway, JAK/STAT3, was not activated on GnRH neurons when leptin was injected into the hypothalamus of women rodent brains. This evidence suggests that an intermediate hormone(s) acting between leptin and GnRH is (are) needed for energy regulation and reproductive signaling to take place. One of the proposed intermediate hormones is KiSS, particularly because leptin controls KiSS mRNA concentration and KiSS directly stimulates the secretion of GnRH (Figure 1.2).

Leptin's involvement in the production of KiSS mRNA is demonstrated through a number of studies using both animal and human models. First, leptin receptors and KiSS neurons are both found in the ARC, providing an environment that is conducive for cell signaling (Rometo et al., 2007). Also, KiSS neurons in the ARC of ewes, mice and humans, contain OB-Rb (Backholer et al., 2010; Smith et al., 2006).

Second, there is a relationship between circulating leptin levels and KiSS mRNA concentrations. In fasting states when circulating leptin levels are low, there is also a significant decrease in KiSS levels (Luque et al., 2007). After only 12 hours of fasting KiSS mRNA levels in rodents are significantly decreased compared to controls. This decline has been shown to persist up to 48 hours (Luque et al., 2007). In addition, when exogenous leptin was injected into the hypothalamus of lean (restricted diet over 6 to 10 months) OVX ewes, there was a significant increase in KiSS gene expression compared to ad libitum fed ewes (Backholer et al., 2010). Lastly, women rats that were fasted for 72 hours demonstrated significantly decreased leptin levels and kisspeptin gene expression in the ARC (Matsuzaki et al., 2011).

Utilizing the leptin deficient mouse model (*Ob/Ob*) has also provided substantial evidence of leptin's relationship with KiSS. Smith et al. (2006) showed a 35% decrease in KiSS positive cells in *Ob/Ob* mice compared to controls. Since *Ob/Ob* mice possess a mutation in the leptin gene, rendering it unable to signal properly, this finding would suggest that leptin may be responsible for KiSS mRNA production. In the same study, injections of exogenous leptin into the hypothalamus of *Ob/Ob* mice caused a significant increase in KiSS mRNA (Smith et al., 2006). Similar to Smith et al., (2006), Quennell et al., (2011) was also able to show that leptin deficient rats presented with decreased KiSS gene expression in the ARC compared to controls. This data also provides supporting evidence for leptin's control of KiSS production.

Another line of evidence to support leptin's control of KiSS mRNA is demonstrated when leptin is injected into rodent and human cell lines. Using a hypothalamic mouse cell line,

exogenous injections of leptin caused significant increases in KiSS mRNA (Luque et al., 2007). Although there is limited human leptin and KiSS data, Morelli et al. (2008) showed significant increases of KiSS mRNA in fetal human neuroblast cells, which were injected with exogenous leptin. It has not been determined though, what specific signaling pathways in both human and animal models, are activated by leptin to increase KiSS mRNA.

Leptin deficiency, as determined by mutations in the leptin gene or OB-Rb gene, also suggests a strong relationship between leptin and reproduction. The ensuing hypogonadotropic hypogandism that results from leptin deficiency causes delayed pubertal development and infertility (Strobel et al., 1998; Clement et al., 1998). Subsequent treatment of leptin deficient individuals with exogenous leptin corrects the abnormal GnRH levels and restores puberty (Farooqi et al., 1999).

Despite these observations, leptin's role in reproduction is described as "permissive", since only small amounts of leptin are needed to correct this delayed pubertal development. Moreover, women who have lipoatrophic diabetes (low fat and low leptin levels) have been shown to have normal reproductive function (Andreelli et al., 2000; Moschos, Chan, Mantzoros, 2002; Hausman et al., 2012).

2.3.2 Additional Metabolic Hormones

There are four other metabolic hormones that are controlled by leptin and that may be involved in metabolic functions that regulate KiSS expression; NPY, POMC, ghrelin and adiponectin. This undoubtedly makes the relationship between leptin and KiSS even more complicated.

Limited studies on reproduction show NPY may decrease fertility and POMC may increase fertility; however the specific link between these hormones and reproduction is still not fully understood (Clarke, Backholer, Tilbrook, 2005; Li, Chen, Smith, 1999; Watanobe, Schoth,

Wikberg, Suda, 1999). NPY 1-Receptors found on select GnRH neurons in the brains of rats, suggests the signaling pathway needed for GnRH stimulation or inhibition is present (Li et al. 1999). Also, Clarke et al., (2005) demonstrated that NPY delayed LH secretion in E_2 induced OVX ewes. POMC may also act as an intermediate hormone between leptin signaling and GnRH activation. Backholer and colleagues (2010) showed that infusion of a melacortin agonist significantly increased LH concentrations in lean ewes. Not only have links between these hormones and different markers of reproduction been shown, more specific links to reproduction, including relationships with KiSS have recently been demonstrated.

Research specifically examining the relationship between KiSS, NPY and POMC has demonstrated that both NPY and POMC neurons are in close proximity to KiSS neurons in the ARC of ewes and vice versa (Backholer et al., 2010). Injections of NPY into mice hypothalamic cell lines are accompanied by significant increases in KiSS mRNA, suggesting NPY may have a positive impact on reproduction (Luque et al., 2007). POMC activation may be partly controlled by KiSS. Fu and van den Pol (2010) conducted a study that showed KiSS strongly innervating POMC neurons. These results suggest there is the potential for reciprocal activation between POMC, NPY and KiSS neurons and the metabolic and reproductive functions they serve.

Ghrelin is a hormone primarily produced in the gut. Circulating levels of ghrelin change based on energy intake and expenditure (Morris & Hansen, 2009). In high energy states circulating ghrelin levels are low, however in low energy states ghrelin levels increase. It is suggested that increases in ghrelin may help to increase NPY concentrations in low energy states (Morris & Hansen, 2009). It is also of note that ghrelin has recently been implicated in controlling LH secretion. Specifically, elevated levels of ghrelin can impair LH secretion, while in a specific dose response manner ghrelin has also been shown to increase LH secretion (Repaci, Gambineri, Pagotto, Pasquali, 2011). Increased ghrelin may also down regulate KiSS mRNA in the medial preoptic area, but not in the ARC of women rats (Forbes, Li, Kinsey-Jones,

O'Bryne, 2009). These contradicting results, suggest further investigation of ghrelin and its role in reproduction is needed.

Like leptin, adiponectin in an adipokine whose circulatory levels are influenced by body mass. Adiponectin levels decrease as fat mass increases, and increase as fat mass decreases (Lihn, Pedersen, Richelsen, 2005). In relation to reproductive function adiponectin may regulate the secretion of reproductive hormones through KiSS. This is supported by research that has shown that when adiponectin is injected into hypothalamic cell lines production of KiSS mRNA levels are significantly inhibited (Wen et al., 2012).

KiSS production and secretion is controlled by a number of reproductive and metabolic hormones (Figure 2.3). It is very apparent though, that further research is needed given the many conflicting results and limited available human data. The investigation of leptin is most critical because of its well-documented links to obesity, reproduction and infertility as well as its known control over a number of other metabolic hormones that affect KiSS concentrations.

2.4 Obesity & Infertility

Adipose (fat) tissue, which is increased in an obese state, is composed of lipid storage cells called adipocytes. Adipocytes are responsible for the formation and storage of adipose tissue via conversion of either glucose or fatty acids and glycerol (Gesta & Kahn, 2009). It is also important to note that adipose tissue functions as an endocrine organ. As mentioned, adipose tissue produces and secretes hormones, such as leptin, which are involved in a number of different functions including satiety and reproduction (Gesta & Kahn, 2009).

In normal weight individuals there is a balance between lipogenesis (fat storage) and lipolysis (fat break down), however in obese individuals there is a disruption in this balance as there is an increase in lipogenesis compared to lipolysis (Gesta & Kahn, 2009). As adipose tissue increases there is also a disruption to hormones released from it. In the case of leptin there is a

significant increase in circulating leptin levels in obese individuals compared to normal weight individuals, which is suggested to impact satiety and reproductive functions (Hausman et al., 2012, Myer et al., 2012).

BMI is a common method by which an individual can be classified as healthy, overweight or obese (Nieman, 2011). BMI is a weight for stature measurement and is calculated by dividing weight in kg by height in meters squared (kg/m^2) (Nieman, 2011). A BMI between 18.0 kg/m^2 and 24.9 kg/m^2 classifies an individual as being a healthy weight. Obesity is classified as a BMI equal to, or greater than 30 kg/m^2 and is broken down into three specific sub-classes: obese class I (30 $kg/m^2 \leq$ BMI $\leq$ 34.9 kg/m^2), obese class II (35 $kg/m^2 \leq$ BMI $\leq$ 39.9 kg/m^2) and obese class III (BMI $\geq$ 40.0 kg/m^2) (Nieman, 2011).

Although BMI is a very common method used to categorize individuals as obese it provides an inaccurate picture as specific body composition components such as fat mass (FM) and lean mass (LM) are not taken into consideration (Wells & Fewtrell, 2006). There are other tools available including skinfolds, bio-electrical impedance (BIA), dual energy x-ray absorptiometry scans (DXA) and multicomponent models, which provide a more detailed description of body composition components and thus may be more useful in identifying individuals who are obese due to an increase in FM (Wells & Fewtrell, 2006). Percent body fat (%BF) is predicted via the use of equations and the measurements obtained from skinfolds (Wells & Fewtrell, 2006). BIA is a non-invasive tool that uses a weak electrical current to estimate FM, LM and %BF (Wells & Fewtrell, 2006). DXA is a whole body x-ray scan that uses high and low photon beams to determine the densities of LM, FM, %BF and aerial bone density (Ellis, 2000). Multicomponent models are considered the gold standard for measuring body composition and used a number of different techniques in combination which include, DXA, densitometry and deuterium dilution to determine %BF, FM and LM (Wells & Fewtrell, 2006).

Women who are obese are three times more likely to experience infertility (Rich-Edwards et al., 1994) as well as have an increased time to conception (Gesink Law, Maclehose, Longnecker, 2007; Wise et al., 2010). The relationship between obesity and reproduction is still unclear; however research suggests abnormal changes in metabolic and reproductive hormones, such as leptin and KiSS may play a role (Evans & Anderson, 2012). Obesity also increases the risk factors for conditions that are contraindications for conceiving such as type II diabetes and/or insulin resistance (Livshits, Seidman, 2009; Moran et al., 2011).

Currently 11 to 15% of Canadian women are infertile and this statistic has significantly increased from 1984 and 1992 where only 5.4% and 8.5% of women respectively, where categorized as infertile (Bushnik et al., 2012; Balakrishnan & Fernado, 1993; Dulberg & Stephens, 1993). One reason for the increased infertility rate may be that women are conceiving later in life (Busnik et al., 2012). However, it is also important to highlight that the number of obese Canadian women has also significantly increased from 7.1% in 1981 to 12.4% of the population from 2007-2009 (Statistics Canada, 2013c).

It has been well documented that as the age of the mother increases so do complications pertaining to conception (American Society for Reproductive Medicine, 2008b). In the 1960's the average age of conception for women in Canada was 23.2. Today, that number has risen to 30.2 years (Milan, 2013). However, this increase in age is not the only reason for increased infertility. Women 18 to 29 years of age experienced an infertility rate of 4.9% in 1984 and in 2009/2010 the rate was between 7 - 13.7% (Bushnik et al., 2012; Balakrishnan & Fernado, 1993). Women 40 - 44 years of age presented with a 4.6% infertility rate in 1984 and in 2009/2010 the rate had risen to between 14.3 – 20.7% (Bushnik et al., 2012; Balakrishnan & Fernado, 1993). Given that this rise in infertility has occurred in parallel to increasing rates of obesity in reproductive aged women is difficult to ignore the potential of at least an important correlative relationship between obesity and infertility.

2.5 Exercise

2.5.1 Exercise, Obesity & Leptin Resistance

Exercise is defined as scheduled, repeated bouts of movement that lead to improvements in an individual's physical fitness (Nieman, 2011). Exercise is recommended as a treatment for obese individuals because there are a number of associated health benefits, including reduction in all cause mortality, decreased risk of developing coronary heart disease, stroke, diabetes, and cancer (colon and breast) (Garber et al., 2012). Also, research demonstrates that exercise is associated with a decrease in elevated leptin levels, such as those demonstrated in a leptin resistant state (Bouassida et al., 2010). It is important to note as well, that exercise has shown to decrease time to conception in obese women (Wise et al., 2012).

There are numerous studies that have investigated the effect of exercise programs on body composition and circulating leptin levels in obese reproductive aged women (Table 2.5). Polak et al. (2006) found that weight, FM, BMI and circulating leptin levels significantly decreased in obese women (n=25) after a 12-week exercise intervention involving cycling 5-days a week at 50% of their voluntary maximum uptake ($VO2_{max}$) for 45 minutes a session. After a 28-week intervention involving treadmill, cycle ergometer, skipping and resistance exercises, 3 to 4 times a week at 60-70% heart rate reserve (HRR), Kondo et al. (2006) found that obese women (n=8) demonstrated a significant decrease in weight, BMI, %BF, FM and circulating leptin levels. Sari et al. (2007) also demonstrated a significant reduction in BMI and circulating leptin levels in obese women (n=23) who participated in an exercise intervention. The exercise intervention involved walking for 4 weeks, 5 times a week at 65-75% heart rate max for 45 minutes a session. Finally, in 2012, Azizi showed that BMI and plasma leptin levels significantly decreased in obese women (n=12) who took part in an 8-week program that involved 3-days a week of aerobic exercise at 65-80% heart rate max for 60 minutes each session, compared to the control group.

Table 2.1 Studies examining circulating leptin levels after an exercise intervention in obese reproductive aged women.
Abbreviations: voluntary maximum oxygen uptake ($VO2_{max}$), heart rate reserve (HHR), heart rate max (HRmax), standard deviation (SD), kilojoules (kj)

Reference	Frequency of Exercise	Intensity of Exercise	Duration of Exercise Session (minutes)	Mode of Exercise	Leptin Levels Before/After (ng/ml)		Body Weight Before/After (kg)		BMI (kg/m^2) Before/After	
Polak et al., 2006*	12 weeks 5 X week	50% $VO2_{max}$	45	Cycle Ergometer	24.3±8.7	18.1±8.3	88.5±8.2	83.3±7.7	32.2±2.2	30.4±2.4
Kondo et al., 2006*	28 weeks 3-4 X week	60-70 % HRR	30	Treadmill, Cycle Ergometer, Skipping	16.4±4.6	12.3±5.4	72.5±6.9	64.5±4.1	29.5±2.7	26.3±5.1
Sari et al., 2007*	4 weeks 5 X weeks	60-80% HRmax	45	Walking	59.1±20.1	51.2±20.5	-	-	40.7±6.7	-
Azizi, 2012*	8 weeks 3 X week	65-80% HRmax	60	Aerobic training	25.68±18.4	13.95±5.3	-	-	32.94	31.65
Kraemer et al., 1999	9 weeks 3-4 X week	1256kj expended per session	20-30	Step aerobic, treadmill, cycle ergometer	28.0 (2.13)	31.04 (2.71)	87.85 (3.50)	86.54 (3.37)	32.48 (1.33)	31.93 (1.79)
Volpe et al., 2008	36 weeks 3-5 X week	-	30	NordicTrack indoor skiing apparatus	Results for women with BMI ≥ 30.0 kg/m^2 Leptin: -20.56±46.0% reported as percent change No change in fat mass or percent body fat					
Arikawa et al., 2011	16 weeks 5 X week	70-85% HRmax	45	Weight bearing aerobic exercise	Results for women with BMI ≥ 30.0 kg/m^2 Leptin: -1.37±1.9% (p=0.967) reported as percent change Weight: No changes between exercise & control group (p=0.11) Body fat: Exercise group lost more compared to control group (p=0.0005)					

*

*Indicates a significant difference in leptin levels from pre to post exercise intervention

Not all studies however have exhibited similar results (Table 2.5). There was no significant decrease weight, BMI, %BF and circulating leptin levels in obese reproductive aged women (n=30) who were involved in a 9-week aerobic exercise program (3-4 days a week for 30-minutes a session) (Kraemer et al., 1999). Similarly, Volpe et al. (2008) found that women (n=46) in a 36-week program consisting of 3 to 5 days a week of indoor NordicTrak skiing failed to demonstrate a significant decrease weight, BMI, %BF and leptin concentrations at the end of the intervention. Finally, there was no significant decrease in circulating leptin levels in obese women (n=41) after 16 weeks of aerobic exercise, that involved exercising 5-days a week at 70-85% heart rate max (Arikawa et al., 2011). However participants did experience a small but significant decrease in percent change of %BF over the study (Arikawa et al., 2011).

When comparing these conflicting results, it becomes apparent that the discrepancy in findings may occur as a result of differences in the frequency, intensity, type and/or duration of each exercise intervention. Also, many studies failed to include a control group, and/or did not control for fasting or timing of menstrual cycles when leptin was sampled. Studies conducted by Azizi, (2012), Sari et al. (2007) and Volpe et al. (2008) all failed to draw blood samples from participants after a 10-12 hour fast. Leptin levels acutely change based on the nutritional content of food ingested; therefore a 10-12 hour fast is needed to obtain accurate circulating leptin levels (Soulis & Kitraki, 2011). Also, all studies listed in table 2.5, except Arikawa et al. (2011), failed to control for the timing of each participant's menstrual cycle when drawing blood. Leptin levels fluctuate during the menstrual cycle (Lugwig, Klein, Diedrich, Ortmann, 2000), therefore it is imperative that blood is drawn at the same time during each cycle. Lastly, the only studies to use control groups were Azizi (2012) and Akrikawa et al. (2011). This lack of uniformity among studies makes it difficult to determine the true effect of exercise on leptin levels.

Although the effect of exercise interventions on circulating leptin levels are conflicting, speculations have been made as to why some studies experience decreases in leptin levels and other have not. Leptin is produced and secreted from adipose tissue (Lee & Fried, 2009). Thus it

has been suggested that a decrease in adipose tissue often measured as weight, %BF, fat mass FM or BMI, is needed to see a decrease in leptin levels (Kraemer et al., 1999; Volpe et al., 2008; Airkawa et al., 2011). When consulting the studies in Table 2.5 all studies that demonstrated a significant decrease in leptin level also has significant decreases in in weight, %BF, FM, or BMI. For example Polak et al. (2006) experienced a significant decrease in weight and FM in addition to decreases in circulating leptin levels. Kondo et al. (2006) also experienced significant decreases in %BF, weight, BMI and FM, while still presenting with a significant decrease in leptin. Finally, Sari et al. (2007) and Azizi (2012) both has significant decreases in BMI and leptin levels.

When compared to studies that did not see a significant decrease in leptin levels, two out of the three studies did not experience significant decreases in body composition. Kraemer et al. (1999) and Volpe et al. (2008) both failed to show significant decreases in weight, BMI and %BF in addition to no changes in leptin. Arikawa et al. (2011) did show a significant decrease in %BF, however it was suggested that the magnitude of the decrease (-0.96%) was not large enough to lead to decreased leptin levels. However, further studies are needed to confirm the premise that a decrease in adipose tissue, leads to a decrease in leptin levels.

2.5.2 Exercise, Weight loss & Infertility

Exercise is a treatment also prescribed to obese women who are attempting to conceive, since a change in body composition (weight, BMI) has been associated with the correction of abnormal menstrual cycles and subsequent conception (Moran et al., 2011). Even a small decrease in body weight (2-5%) is associated with increases in regular ovulation, spontaneous pregnancy and correction in abnormal reproductive hormone levels (Moran et al., 2011). In a study conducted by Harlass, Plymate, Fariss, Belts (1984) involving six obese women, who lost between 7.0 – 18.8% of their body weight, all resumed regular menses as well as experienced

correction in abnormal reproductive hormones. In two similar studies, significant improvements in ovulation and spontaneous pregnancies were demonstrated in obese infertile women who lost between 6.2 and 10.2kg/m^2 in their BMI, respectively (Gallentley, Clark, Tomlinson, Blaney, 1996; Clark et al., 1998). Finally, Hollmann, Runnebaum and Gerhard (1996) demonstrated a correction of abnormal menstrual cycles in obese women who had oligomenorrhea or amenorrhea and lost even a small portion of their body weight (5.6±3.4 kg).

Although all four studies listed above did not employ the use of exercise or physical activity to decrease body composition (all studies used diet interventions), weight loss through exercise could potentially produce similar results as well as have the added benefit of increasing cardiovascular and musculoskeletal health.

2.5.3 Exercise & Kisspeptin

Currently, there are no published studies that have examined whether KiSS concentrations are altered after an acute bout of exercise or after a chronic exercise intervention. It is important that this gap in the literature be addressed to fully understand the interplay between obesity, infertility and exercise.

2.6 Summary of Literature Review

Infertility is a devastating health issue that not only affects a women's ability to conceive but is also associated with decreased health related quality of life scores (Chachamovich et al., 2010) and the potential for increased financial strain (ARTUS Fertility Centre, 2013). Coinciding with the increase in infertility is there is an increase in Canadian reproductive aged females as well (Stat Canada, $2013_{a,b}$).

Researchers are investigating mechanisms that might cause infertility in obese states. Results thus far suggest changes in metabolic and reproductive hormones, including leptin and

KiSS, may affect reproductive function. Specifically, elevated leptin levels may impact cell signaling to KiSS, the hormone responsible for increasing GnRH secretion. One way to reduce elevated leptin levels is through long-term exercise interventions; however, changes in KiSS after an exercise intervention have yet to be studied.

Thus the **purpose** of this study was to determine the effects of a l2-week progressive aerobic exercise intervention on FM and %BF, as well as circulating leptin and KiSS levels in obese women. It was **hypothesized** that obese women who were randomized into a 12-week progressive aerobic exercise intervention would show a decrease in FM, %BF and circulating leptin levels and an increase in circulating KiSS levels from baseline testing to mid-point testing and from mid-point testing to end-point testing. There would be no change in FM, %BF, circulating leptin or KiSS levels over the 12-week period in women randomized into the control group.

3.0 METHODS

3.1 Study Design

A randomized controlled experimental design was used for this study. Participants were randomized, one to one, into either a 12-week exercise intervention group or a 12-week non-exercise control group, by a block design protocol after initial screening and baseline testing. A block design protocol was used to ensure there were an equal number of participants in each group.

3.1.1 Exercise Intervention Protocol

Individuals in the exercise intervention group completed a 12-week supervised progressive aerobic exercise program. Participants walked on a treadmill 4-days a week for 12-weeks. Participants exercised under the supervision of a Canadian Society for Exercise Physiology - Certified Exercise Physiologist (CSEP-CEP) at the gym in R.J.D. Williams Building (Williams gym) located at 221 Cumberland Avenue North, Saskatoon, Saskatchewan for at least 3 of the 4 sessions each week. Participants had the choice to perform the 4^{th} session at the Williams gym or unsupervised at a location of their choice. If participants did perform the 4^{th} session at a location of their choice they were given a training log (APPENDIX A) to record their sessions. The training log was handed in at the completion of the study. Participants used a separate training log to track their exercise preformed at the Williams gym (APPENDIX B).

Each exercise session, supervised or unsupervised, included a 10-minute warm up at a speed that increased the participant's heart rate to 40% of their predicted maximum heart rate (PMHR). The equation used to determine each participant's PMHR was 220 minus current age. The warm-up was followed by 30 minutes of walking between 65-75% PMHR. The session ended with a 10 minute cool down period, walking at a speed that brought the participant's heart

rate back down to 40% PMHR. Every two weeks the time spent walking between 65-75% PMHR increased by 5 minutes to a maximum of 55-minutes by the end of week 12.

Heart rate (HR) was measured each session using a heart rate monitor and watch. HR was recorded to determine compliance of the participants to the designated target exercise HR (Polar Team 2) (APPENDIX C). Participants were taught how to take their radial pulse to ascertain their intensity when performing the 4th exercise session at a location different from the Williams gym. Adherence to the intervention was met if participants in the exercise intervention group attended a total of 85% of all exercise sessions.

Participants in the exercise intervention group were asked to adhere to specific instructions over the period of the study, including not performing any exercise, which was defined as any scheduled and repeated movement activity, outside the study protocol. If participants did perform extra exercise that was outside of the study protocol, they were asked to record the frequency, intensity, time and type of the exercise in a journal (APPENDIX D), which was submitted to the researcher at the end of the study.

3.1.2 Non-exercise Control Group Protocol

Individuals in the non-exercise control group were asked to continue their current lifestyle behavior and refrain from starting any exercise during the course of the study. However, if exercise was performed they were asked to record the frequency, intensity, time and type of exercise in the same journal used for the exercise group (APPENDIX D) and then submit it to the researcher at the end of the study.

3.2 Participants

Ethics approval from the University of Saskatchewan Ethics Board was obtained prior to the start of recruitment. Sixteen women participants were recruited from local doctors' offices, ads posted across the University of Saskatchewan campus (APPENDIX E) and in the Saskatoon Express newspaper.

There was no data available in the literature on circulating plasma KiSS levels after an exercise intervention; therefore the power calculation for an 80% type two-error was based on average changes in circulating leptin levels after exercise interventions in obese women (Polak et al., 2006; Kondo et et al., 2006; Azizi, 2012) (APPENDIX F).

To be included in the study women had to be between the age of 18 and 45 and be obese according to the WHO classification of a BMI $\geq$ 30.0 kg/m^2. In Saskatchewan, the current number of overweight and obese women is almost 200,000 (Stats Canada, 2014). Participants had to be weight stable for the two months prior to the start of the study. This "weight stable" condition was necessary since a loss in body weight is associated with decreases in circulating plasma leptin concentrations (Bouassida et al., 2010).

Participants needed to be sedentary or have low exercise levels for the two months prior to the start of the study. Exercise levels were controlled for as any changes in total body energy expenditure (decrease in body weight) could have affected circulating plasma leptin concentrations (Hilton & Loucks, 2000).

Exclusion criterion included; pregnancy, a non-regular menstrual cycle, any reproductive disorders, such as Polycystic Ovary Syndrome or hyperandrogenism and use of hormone contraception two months prior to the start of the study. Leptin is not affected by hormone contraception however, it is known that KiSS mRNA concentrations are affected by E_2 (Fallah Pour, Chadegani, Korani, 2012; Smith et al., 2005). Therefore, any potential participants who were using hormone contraception were excluded from the study. If participants became

pregnant, experienced abnormal menstrual cycles (>35 days), started hormone contraception during the study or voluntarily dropped out of the study all their data was excluded. These data was excluded as proper analysis would be difficult to preform and results obtained may obscure true results.

Potential participants were also excluded if they reported being diagnosed as insulin resistant or diabetic (type I or II). Finally potential participants were excluded if their Glycated Hemoglobin test (HbA1c) result (screening procedure) was 5.7% or greater, indicating undiagnosed insulin resistance or diabetes. Individuals diagnosed with insulin resistance or diabetes (type I of II) were excluded because the hormone insulin can alter circulating leptin levels (Lee & Fried, 2009).

3.3 Screening Protocol

Prior to the start of the study, the researcher screened all potential participants. Screening procedures took place at the Physical Activity Complex (PAC) in room 359 (222 87 Campus Drive, Saskatoon, Saskatchewan) and the Royal University Hospital (RUH) (103 Hospital, Saskatoon Saskatchewan). The screening procedures done at the PAC took place in a single visit and included signing the consent form and completing the Physical Activity Readiness Questionnaire Plus form (PAR-Q+), 2-Month Physical Activity Questionnaire (2-Month PAQ-AD) and the Self-report Menstrual & Medical History Questionnaire. Anthropometric measurements, including height, weight and waist circumference were also taken at this time. If the participant met all screening criteria done at the PAC they were then sent to the RUH to have one final screening test performed, a Glycated Hemoglobin test (HbA1c).

Initial screening at the PAC began with the researcher ensuring participants understood the consent form (APPENDIX G) by going through the ‘Consent Form – Understanding Check List’ (APPENDIX H). After the researcher and potential participants finished the Consent Form

– Understanding Check List, the potential participant was asked to sign the consent form. If the potential participant was not ready at that time to sign the consent form they were able to contact the researcher if they eventually did decide to sign it.

During the signing of the consent form, participants were also asked if they wanted to sign a photo release form (attached to consent form). The researcher explained to participants that throughout the study the researcher would be taking photos of the participants for future use in scientific journals, presentations or posters, however all photos would have any personal identifiers, including faces and tattoos, blurred out.

After the consent form was signed, potential participants completed PAR-Q+, 2-Month PAQ-AD and the Self-report Menstrual & Medical History Questionnaire as well as had height, weight and waist circumference measured. Height and weight were used to calculate BMI.

3.3.1 Physical Activity Readiness Questionnaire Plus Form (PAR-Q+)

The PAR-Q+ (CSEP, 2012) (APPENDIX I) is a physical activity clearance tool created by the Canadian Society for Exercise Physiology (CSEP). The PAR-Q+ is administered to individuals who are starting an exercise program to screen them for any potential contraindications to exercise prior to starting.

If the PAR-Q+ was filled out with a positive response, potential participants were asked to have a Physician Physical Activity Readiness Clearance form signed by their doctor clearing them for unrestricted physical activity before continuing on with the screening procedures (APPENDIX J) (CSEP, 2014).

3.3.2 Anthropometric Measures

Potential participants had their height and weight measured to calculate their BMI and waist circumference to obtain a measure of central adiposity. Height was measured with a stadiometer (Holtain Ltd, Crymych, Dyfed, UK) to the nearest 0.1 cm and weight was measured with a physician's scale (Toleda Scale Company, Windsor, Ontario, Model # 2830) to the nearest 0.1kg. BMI (kg/m^2) was calculated by dividing weight (kg) by height (m^2). To measure waist circumference, potential participants were instructed to stand with feet hip width apart and arms crossed against their chest. Using an anthropometric tape measure, the researcher took the waist circumference measurement at the top of the potential participant's iliac crest at the end of the participant's normal exhalation. The measurement was taken to the nearest 0.1 cm (CSEP, 2013).

3.3.3 2-Month Physical Activity Questionnaire (2-Month PAQ-AD)

The 2-Month PAQ-AD (APPENDIX K) was completed to determine physical activity/exercise levels of potential participants in the two months prior to the start of the study (Copeland, Kowalski, Donen, Tremblay, 2005). The 2-Month PAQ-AD was adapted from the 7-Day Physical Activity Question for Adults (7-Day PAQ-AD), which is used to establish physical activity/exercise levels of an individual over the prior seven days. To fit the current study need of determining the physical activity/exercise level of participants in the two months prior to the study the question, "Are the responses you made on the PAQ-AD representative of your physical activity behavior in the prior two months?" was added as the final question to create the 2-Month PAQ-AD. Potential participants were asked to respond either yes or no to the question. Those who responded "yes" met the physical activity inclusion criteria of the study provided the 2-Month PAQ-AD indicated them as having a low physical activity level (level 3 or below).

The 7-Day PAQ-AD uses a likert type scale to answer questions pertaining to the type and frequency of activities performed during the prior week. The validity of the 7-Day PAQ-AD to other measurements of physical activity range from r=0.56 to 0.63 (Copeland et al., 2005).

3.3.4 Menstrual & Medical History Questionnaire

Potential participants completed the Menstrual & Medical History Questionnaire (APPENDIX L) to screen for reproductive disorders, abnormal menstrual cycles, use of hormone contraceptives and medical disorders such as diabetes or insulin resistance.

3.3.5 Glycated Hemoglobin (HbA1c)

If all inclusion criteria up to this point had been met, potential participants were then given a blood requisition form for the RUH to have a HbA1c performed. The HbA1c test was conducted to ensure potential participants were not diabetic (type I or II) or insulin resistant. The HbA1c test is an indirect method of measuring the average blood glucose of an individual over the past three months and is also used to diagnose diabetes (Borai et al., 2011; American Diabetes Association, 2014). Any participants who had an HbA1c reading of 5.7% or greater were excluded from the study, as this is the level used to classify individuals as insulin resistant (American Diabetes Association, 2014). The test involved having a 7ml vacutainer of blood drawn by venipuncture from a vein at the inside of the elbow by a registered RUH phlebotomist or registered nurse. The blood was then analyzed in the blood chemistry laboratory at RUH to ascertain HbA1c level.

3.4 Testing Protocol

Testing was completed at three time points; prior to the start of the intervention (baseline), at the mid-point (week 6-8) of the intervention, and within 48-72 hours of completing the intervention. Every effort was made to test all participants between days 6 - 9 of their menstrual cycle (follicular phase) since leptin levels do change throughout the reproductive cycle (Ludwig et al., 2000) and KiSS levels are affected by fluctuating E_2 levels during the menstrual cycle (Smith et al., 2005). However, due to circumstances out of the researcher's control, some participants were tested between days 6 – 15 of their menstrual cycle. Testing procedures were preformed at the PAC (rooms 344 & 349) and the Williams gym. Measurements taken at each testing session included anthropometric measures (height, weight & waist circumference), blood draws for later measurement of leptin and KiSS, predicted maximal oxygen uptake, 3-day dietary recall, a 7-day PA-AD and body composition (percent fat mass, fat tissue [kg] and lean tissues [kg]) by a Dual-energy X-ray absorptiometry scan (DXA) for both the exercise and non-exercise control group.

3.4.1 Pre-testing Procedures

Participants were asked to follow specific pre-test procedures starting 24-hours prior to testing. Acute food intake causes acute changes in leptin; therefore it was imperative to standardize food intake the 24-hours prior to all testing sessions (Soulis & Kitraki, 2011). Standardization of food intake was completed via a 24-Hour Dietary Log (Appendix M). Participants were asked to record all food, liquids and supplements consumed as well as the measurements of each in a logbook for the 24-hours prior to testing. This logbook was then handed to or emailed to the researcher the morning of baseline testing. If participants handed in the logbook, the researcher photocopied it and handed the original back to the participants to refer to for the two future testing points. For the following two testing points (mid & end)

participants were asked to eat the same meals they recorded in the 24-Hour Dietary Log the day before baseline testing and again record it on a blank 24-Hour Dietary Log to hand into the researcher.

3.4.2 Anthropometry

Height was taken by a stadiometer (Holtain Ltd, Crymych, Dyfed, UK) and measured to the nearest 0.1 cm. Weight was taken on a standard Physician Scale (Toleda Scale Company, Windsor, Ontario, Model # 2030) and measured to the nearest 0.1 kg. BMI (kg/m^2) was calculated by dividing weight (kg) by height (m^2). To measure waist circumference, participants were instructed to stand with feet hip width apart and arms relaxed by their sides. Using an anthropometric tape measure, the researcher took the waist circumference measurement at the top of participant's iliac crest and at the end of the participant's normal exhalation. The measurement was taken to the nearest 0.1 cm (CSEP, 2013).

3.4.3 Blood Draws & Blood Plasma Measures

Participants were asked to fast for 10-12 hours prior to the blood draw to standardize all hormone levels; if participants came in for testing at 7:00 they were asked to stop eating between 19:00 and 21:00 the day before. A trained phlebotomist performed a single venipuncture at the antecubital space of the left or right elbow of participants for each blood draw. Three 4ml ethylenediamineteraacetic acid (EDTA) coated vacutainers were used to collect participant's blood at each testing session. The phlebotomist inverted the vacutainers 8 to 10 times and then the vacutainers were stored in a fridge (4 degrees Celsius) in the bio-hazardous laboratory (room 361) in the PAC for 20 to 30 minutes until the rest of the testing was completed.

Once all other tests were completed (predicted maximal oxygen uptake, the 3-day dietary recall and 7-day PA-AD) the vacutainers were removed from the fridge and the blood was

pipetted into centrifuged tubes along with 100µl of aprotinin per every 1 ml of blood collected (Phoenix Pharmaceuticals, 2014). The tubes were then centrifuged for 15-minutes at 4-degrees Celsius to separate and collect the plasma. After the centrifugation was complete all plasma was pipetted into capped centrifuge tubes and kept in a freezer at -80 degrees Celsius until the analysis of circulating leptin and KiSS concentrations were performed (Phoenix Pharmaceuticals INC, 2013).

Once all participants had completed the study all plasma samples were sent from the University of Saskatchewan to Phoenix Pharmaceuticals Inc (Burlingame, California, USA) for analysis of the concentration (in duplicate) of plasma leptin and KiSS. An Enzyme Linked Immunosorbent (ELISA) test was used to determine circulating leptin (sensitivity 0.321 ng/ml) (Kit # EK-003-12) and an Enzyme Immunoassay (EIA) was used to determine concentrations of circulating KiSS (sensitivity 0.08 ng/ml) (Kit # FEK-048-56).

ELISA and EIA all use an antigen/antibody technique to measure the concentration of a hormone in plasma. The hormone of interest is labeled as the antigen and a serum that contains a specific antibody to the antigen is added to the plasma (Crowther, 2009). A colour change is produced based on the amount of antigen that binds to the antibody complex. The change in colour is measured by a spectrophotometer and indicates the concentration of the antigen in the plasma (Crowther, 2009). Leptin and KiSS were measured to the nearest nanogram per milliliter (ng/ml).

3.4.4 Predicted Maximal Oxygen Uptake

The Ebbling Single Stage Walking Treadmill Test was used to measure participant's predicted maximal oxygen uptake. The protocol involved having participants warm up by walking on a treadmill for 4-minutes at zero percent grade and a speed that raised their HR between 50 and 70% of their PMHR. After the 4-minute warm up, participants continued at the

same speed, but the grade of the treadmill increased to 5%. Participants continued to walk for 4 more minutes to ensure steady state HR was reached. If a steady state HR was reached by 4-minutes the test ended and participants cooled down by walking at a reduced speed and zero percent grade. If a steady state HR was not reached by 4-minutes, participants continued for an additional minute. If a steady state HR was not reached at the end of 5-minutes the test was terminated and the participant was asked to return on a subsequent day to perform the test again. Predicted maximal oxygen uptake was determined pre, mid and post exercise intervention to ascertain whether there was any relationship between changes in fitness and changes in circulating KiSS and leptin levels.

3.4.5 7-day PAQ-AD & 3-day Dietary Recall Questionnaires

Participants completed a 7-day PAQ-AD (Appendix N) and a 3-day dietary recall (Appendix O) questionnaire. The 7-day PAQ-AD and 3-day dietary recall questionnaires were completed by participants to determine whether there were any substantial changes to diet or exercise/physical activity routines that may have affected the circulating hormones of interest.

The 7-day PAQ-AD was the same physical activity level questionnaire (2-month PAQ-AD) used during the screening procedures (Copland et al., 2002). However, the question, "Are the responses you made on the PAQ-AD representative of your physical activity behavior in the prior two months?" was eliminated as the question was not relevant for the purpose of the survey.

The 3-day dietary recall questionnaire was similar in format to the 24-hour dietary log. However, participants were asked to recall to the best of their abilities all the food, liquids and supplements they ingested the 3-days prior to testing.

After the anthropometric measures, blood draw, predicted maximal oxygen uptake test and questionnaires were completed participants were provided with granola bars and juice. The

intent of this was to raise their blood glucose levels back to normal before they left the laboratory.

3.4.6 Body Composition

A DXA (QDR Discovery Wi, Hologic, Inc., Beford, Md.) was performed to measure body composition, specifically percent body fat (%BF), fat mass (FM) and lean mass (LM) of participants. The use of the DXA allowed the researcher to see the specific changes in each participant's body composition over the study. The DXA scan was performed in the evening at the Williams gym within 7-days of initial baseline testing and then again within 7-days of post testing.

DXA is a specific x-ray that assesses percent FM, lean mass (LM), and areal bone mineral density (g/cm^2) (Ellis, 2000). High and low photon beams produced from the DXA pass through FM, LM, and bone to determine the densities (Ellis, 2000). Radiation exposure is low and is equivalent to that received on a single transcontinental airplane trip (Lloyd, Eggli, Miller, Eggli & Dodson, 1998).

On the same evening of the baseline DXA scan, participants were also given an orientation to the gym at the Williams Building. The orientation included familiarizing participants with all emergency exits, bathrooms and equipment. Participants were also taken through a 'mini workout session' to familiarize them with the protocol.

3.5 Randomization Protocol

After participants completed all screening and baseline testing protocols they were randomized into either the exercise intervention group or the non-exercise control group. A block randomization protocol was used to ensure each group had equal numbers. Prior to the beginning of the study, the researcher used www.randomizer.org to produce the block-randomized

sequence in which participants would be assigned to after completing all screening and baseline protocol (Appendix P).

3.6 Statistical Analysis

Outliers in data were determined by standard error (SE<±2.0) of skewness and kurtosis. If outliers did present transformations were performed and data was rerun to determine if it was significantly different from the raw data. If the transformed data was not significantly different from the untransformed data, the original raw data was used.

Two participants in the exercise group were missing data points for the mid-point testing 7-day PAQ-AD scores. Both participants had to leave the testing session prior to completing the survey due to unforeseen circumstances (needed to go home and tend to children). The 7-day PAQ-AD questionnaire was sent home with each participant to complete and they were instructed to bring back the completed questionnaire to the researcher at their next appointment, however the participants were not compliant. The missing data points were dealt with by using the same score achieved in the baseline 7-day PAQ-AD for the mid-point 7-day PAQ-AD score.

For descriptive data normal distribution and equality of variance of all measurements were calculated using the Sharpio-Wilk test and Levene's Test for Equality of Variance, respectively. If assumptions of normal distribution and equality of variance were met independent samples T-tests were run to determine if there was a significant difference between baseline measures in the exercise intervention and control group.

Comparisons of weight, BMI, waist circumference (WC), VO2max, 7-day PAQ-AD scores, FM and LM between the exercise and control group were performed. A 2x3 factorial ANOVA was run to see if there was a main effect of group (exercise vs. control), a main effect of time (baseline vs. mid-point vs. end-point testing) or an interaction effect (group vs. time) for weight, BMI, waist circumference, VO2max, 7-day PAQ-AD scores. A 2x2 factorial ANOVA

was run to determine if there was a main effect of group (exercise vs. control), a main effect of time (baseline vs. end-point testing) or an interaction effect (group vs. time) for %BF, FM and LM. If significant interaction effects were found independent T-tests and pairwise comparisons were run to determine where the significant differences were.

To answer the main hypothesis, two stepwise multiple linear regression analyses were run to determine which variables significantly contributed to end-point (a) circulating leptin and (b) circulating KiSS levels. Decreases in circulating leptin levels after an exercise intervention are often found in conjunction with significant decreases in BMI and weight (Polak et al., 2006; Kondo et al., 2006; Sari et al., 2007; Azizi, 2012). Therefore percent change from baseline to end-point testing for BMI and weight along with percent change from baseline to end-point testing for BF, FM, LM as well as age and intervention group status (exercise vs. control) were used in the model. A study by Pita et al., (2011) found that BMI and leptin levels influenced circulating KiSS levels in pre-pubertal healthy and obese girls and girls with idiopathic precocious puberty. Thus, percent changes in BMI and leptin from baseline to endpoint testing were included in the KiSS regression model. Other variables that were included in the KiSS regression model were age, intervention status (exercise vs. control group) and percent changes from baseline to end-point testing for weight, %BF, FM and LM.

After the regression analysis was performed, two 2x3 factorial ANCOVAs were run to calculate if there was a main effect of group (exercise vs. control), a main effect of time (baseline vs. mid-point vs. end-point testing) or an interaction effect (group vs. time) for end point circulating (a) leptin and (b) KiSS levels. The covariates used in the 2x3 factorial ANCOVA were the variables that came back as being significant contributors to their specific regression models. Also, baseline levels of leptin and KiSS were used as covariates in their respective ANCOVAs. If significant interaction effects were found independent T-tests and pairwise

comparisons were run to determine where the significant differences were. All analysis was done using Statistical Package for Social Sciences (SPSS version 22). Alpha was set at $p<0.05$.

Individual participant data was also graphically represented for a number of different variables including weight, %BF and circulating leptin and KiSS levels. Graphical representation of data was created to look for specific trends that may have not appeared in the statistical analysis, due to the small sample size resulting in an underpowered study. Also throughout the study there were drastic changes to exercise attendance by participants in the exercise group (illness or other commitments). Thus graphical representation of specific data may have provided evidence to explain specific results or lack thereof in the data.

4.0 RESULTS

All data was checked for outliers using skewness and kurtosis. No violations were found; therefore the original raw data was used for all analyses.

4.1 Descriptive Data

Twenty seven participants between the ages of 18 and 45 were screened for the present study, however only twenty participants met all screening criteria. Five participants dropped out prior to randomization and a further five dropped out during the intervention. The ten participants left were used for analysis (APPENDIX Q).

All participants were free of use of hormonal birth control, were obese ($BMI \geq 30kg/m^2$) and inactive during the two months prior to the start of the study. Inactivity was defined as scoring a three or less on the 2-month Physical Activity Questionnaire, which was completed during the screening protocol (Copland et al., 2005). Participants were randomly assigned (randomized block design) to either the 12-week exercise intervention group (n=5) or the 12-week control group (n=5). HbA1c scores for all participants were below 5.7%, indicating that

none of the participants presented with pre-diabetes or diabetes. Independent t-tests found no significant differences in age, weight, BMI, WC, %BF, FM, LM, 2-month PAQ-AD scores, VO_2max, and HbA1C between the exercise and control group at the onset of the study (Table 4.1).

Table 4.1 Baseline Participant Data for Exercise and Control Group

Measures	**Exercise (n=5)**	**Control (n=5)**	**Significance (p)**
Age (years)	31.0±6.0	36.0±3.8	0.08
Wt (kg)	97.0±21.3	99.1±14.1	0.06
Ht (cm)	166.7±11.7	164.3±5.9	0.07
BMI (kg/m^2)	34.6±3.9	36.7±4.7	0.46
WC (cm)	108.3±8.6	110.9±7.3	0.42
% BF	46.7±3.3	45.7±3.5	0.75
FM(kg)	45.0±11.9	45.0±8.3	0.13
LM(kg)	48.1±9.2	50.6±7.1	0.30
2-month PAQ-AD Score	1.4±0.5	1.3±0.4	0.29
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	22.7±7.7	27.9±7.4	0.86
HbA1C Score	5.3±0.2	5.2±0.2	0.76

Data are displayed as Mean ± SD
Comparison was made between exercise and control group using in independent t-tests.
Abbreviations: weight (Wt), height (Ht), body mass index (BMI), waist circumference (WC), percent body fat (%BF), fat mass (FM), lean mass (LM), 2-Month Physical Activity Questionnaire (2-Month PAQ-AD), voluntary maximum oxygen uptake (VO_2max), glycated hemoglobin (HbA1C)

4.2 Exercise Group vs. Control Group

4.2.1 Adherence to Exercise Intervention

The average adherence rate was 72.9% (range 58.3% and 100%) for participants in the exercise group.

4.2.2 Anthropometric Measurements

Results for the 2x3 factorial ANOVA for weight, BMI, WC, VO_2max and the 7-day PAQ-AD score are found in Table 4.2. All data was checked for sphericity using Mauchly's test of Sphericity. Two variables, weight, $\chi^2(2)=8.103$, p=0.02, and BMI, $\chi^2(2)=12.668$, p=0.002, violated the assumption. Thus, the Greenhouse-Geisser correction was used instead of the sphericity assumed value in the Within Subjects Effects to determine if there was a significant difference in either weight or BMI.

Table 4.2 Weight, BMI, Waist Circumference, VO_{2max} and 7-day PAQ-AD for the Exercise and Control Group at Baseline, Mid-Point and End-Point Testing

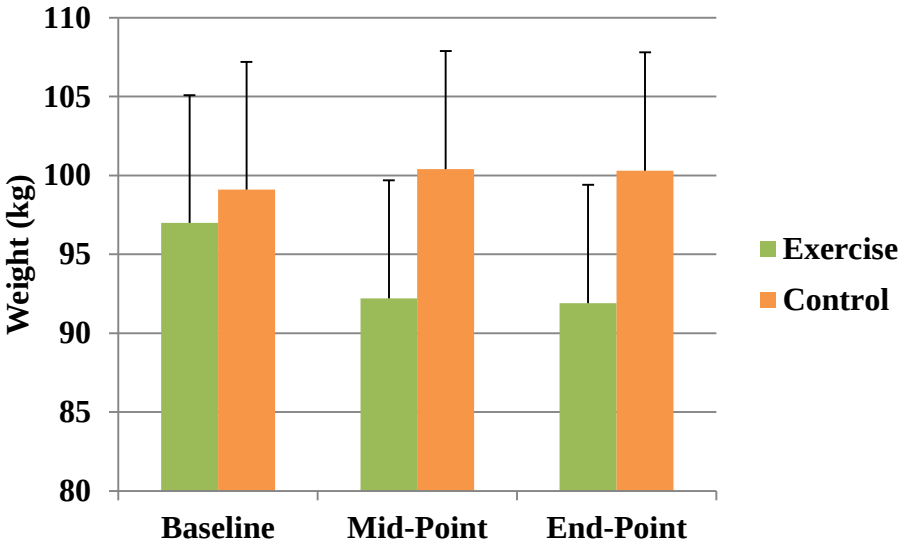
	Baseline Testing		**Mid-Point Testing**		**End-Point Testing**	
Variables	**Exercise Group**	**Control Group**	**Exercise Group**	**Control Group**	**Exercise Group**	**Control Group**
Wt (kg)	97.0±21.3	99.1±14.1	92.2±19.0	100.4±14.1	91.9±19.3	100.3±13.6
BMI (kg/m^2)	34.6±3.7	36.7±4.7	33.1±4.0	37.2±4.7	33.0±4.1	37.2±4.7
WC (cm)	108.3±8.6	110.9±7.3	106.3±10.0	111.9±9.8	104.6±9.3	111.5±7.7
VO_2max ($ml.kg.min^{-1}$)	22.7±7.7	27.9±7.4	34.1±5.4	28.8±2.6	29.3±5.9	29.4±4.5
7-day PAQ-AD Score	1.4±0.5	1.3±0.4	1.7±0.5	1.3±0.4	1.5±0.8	1.6±0.5

Data are displayed as Mean ± SD
Comparisons were made between groups (exercise vs. control) and over time (baseline vs. mid-point vs. endpoint) using a 2x3 factorial ANOVA
Abbreviations: weight (Wt), body mass index (BMI), waist circumference (WC), voluntary maximum oxygen uptake (VO_2max), Physical Activity Questionnaire (PAQ-AD)
*There were no main effects of group or time point

There were no main effects for between group differences (exercise vs. control) (p>0.05) or within group differences (baseline vs. mid-point vs. end-point testing) for weight (p=0.151), BMI (p=0.22), WC (p=0.37), VO_2max (p=0.06) and 7-day PAQ-AD scores (p=0.62). However two interaction effects were found. There was a significant interaction effect of group x time for weight F(1.19,6.80), p=0.02, indicating participants' mean weight changed differently across the exercise and control group and over the three testing time points (Figure 4.1). There was also a

significant interaction effect of group x time on BMI, F(1.10,7.27), p=0.02. This indicated that participants' mean BMI changed differently across the exercise and control group and over the three testing time-points.

Figure 4.1 Estimated Marginal Means and Standard Error of Weight for the Exercise and Control Group at Baseline, Mid-Point and End-Point Testing



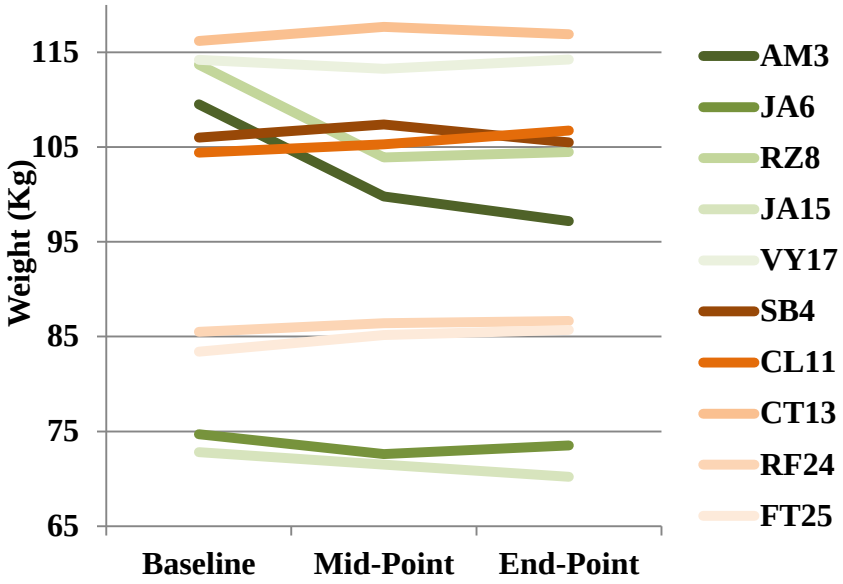
Independent t-tests (between group) did not however reveal significant differences in mean weight between the exercise and control group at baseline testing, t(8) = -0.19, p=0.86, mid-point testing, t(8)= -0.77,p=0.46 or end-point testing, t(8)= -0.79, p=0.46. Levene's Test of Equal Variance was not violated for the three independent t-tests run (p>0.05) (exercise vs. control group at baseline, exercise vs. control at mid-point, exercise vs. control at end-point); therefore the Equal Variance Assumed values were used.

The pairwise comparisons for within group differences across the three testing time points for weight indicated there was a significant increase in mean weight between baseline and mid-point testing for the control group t(4) = -7.63, p=0.01.

Individual participant data for weight is displayed in Figure 4.2. As mentioned individual participant data was graphically represented to look for specific trends in the exercise and control group that may have not presented in the statistical analysis. The sample size was underpowered

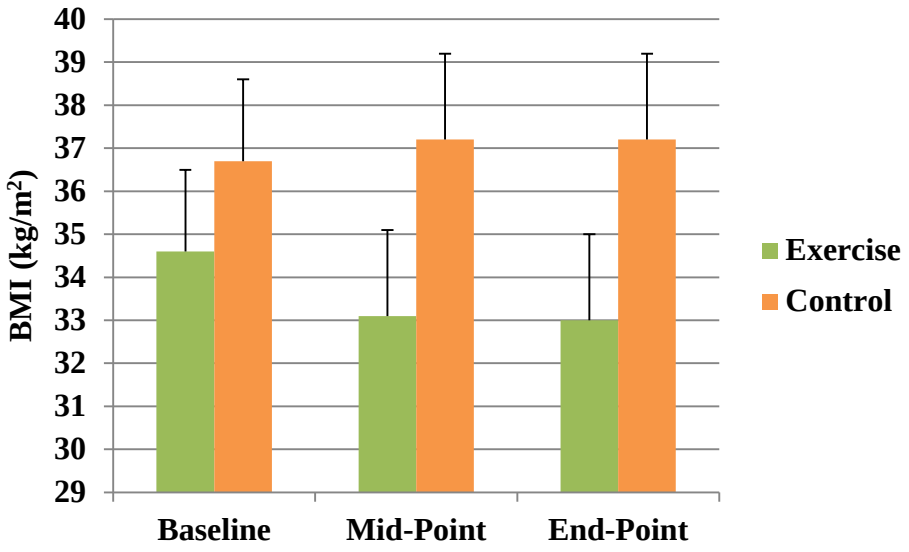
as well the researcher observed large variability among participants, which may have affected a number of variables being studied. Participants in the exercise group displayed a decreasing trend in weight from baseline to mid-point testing. Two out of the five exercise participants continued to decrease in weight from mid to end-point testing, while the other three exercise participants slightly increased (0.55 – 1.24%) in weight. As supported by the ANOVA results, the control group saw a significant increase in weight from baseline to mid-point testing and then a plateau from mid to end-point testing.

Figure 4.2 Individual Participant Body Weight for Baseline, Mid-Point and End-Point Testing
*Green lines represent exercise group & orange lines represent control group



The independent t-tests did not produce significant differences in mean BMI between the exercise and control group at baseline testing, $t(8) = -0.78$, $p=0.46$, mid-point testing, $t(8)= -1.49$,$p=0.18$ and end-point testing, $t(8)= -1.52$, $p=0.17$ (Figure 4.3). Levene's Test of Equal Variance was not violated for the three independent t-tests run ($p>0.05$) (exercise vs. control at baseline, exercise vs. control at mid-point, exercise vs. control at end-point); therefore the Equal Variance Assumed values were used.

Figure 4.3 Estimated Marginal Means and Standard Deviation of BMI for the Exercise and Control Group at Baseline, Mid-Point and End-Point Testing



The pairwise comparisons for within group differences across the three testing time points for BMI indicated there was a significant increase in mean BMI between baseline and mid-point testing for the control group t(4) = -2.56, p=0.01.

Results for the factorial ANOVA for the DXA measurements including %BF, FM and LM are presented in table 4.3. Two interaction effects: %BF F(1, 17.7), p=0.01, (Figure. 4.4) and FM, F(1, 10.8), p=0.01 (Figure 4.5) were detected. This indicates that participants' mean %BF and FM changed differently across the exercise and control group and over all testing points.

Table 4.3 %BF, FM & LM Between the Exercise and Control Group at Baseline, Mid-Point and End-Point Testing

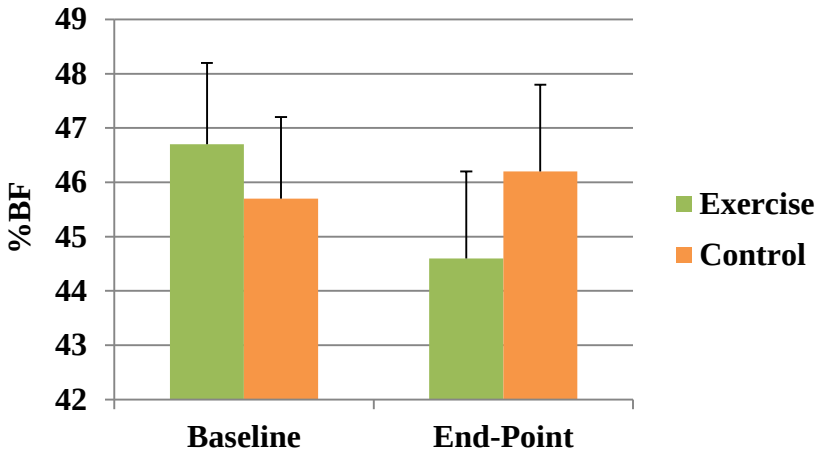
	Baseline Testing		**End-point Testing**	
Variable	Exercise Group	Control Group	Exercise Group	Control Group
%BF	46.7±3.3	45.7±3.5	44.6±3.8*	46.2±3.6
FM (kg)	44.9±11.9	45.0±8.3	41.1±10.8	46.0±8.3
LM (kg)	48.1±9.2	50.6±7.1	48.0±8.4	50.9±6.5

Data are displayed as Mean ± SD
Comparison was made between groups (exercise vs. control) and over time (baseline vs. mid-point vs. endpoint) using a 2x2 factorial ANOVA
Abbreviations: percent body fat (%BF), fat mass (FM), lean mass (LM)
*Significant difference with-group between Baseline & End-point Testing ($p<0.05$)

Independent t-tests (between group) did not reveal any significant differences in mean %BF between the exercise and control group at baseline testing, t(8)=0.43, p=0.68 or end-point testing, t(8) = -0.68, p=0.52. Levene's Test of Equal Variance was not violated for the two independent t-tests (p>0.05) (exercise vs. control group at baseline, exercise vs. control at end-point); therefore the Equal Variance Assumed values were used.

The pairwise comparisons for within group differences across the two testing time points revealed a significant decrease in mean %BF between baseline and end-point testing for the exercise group t(4)=4.71, p=0.01.

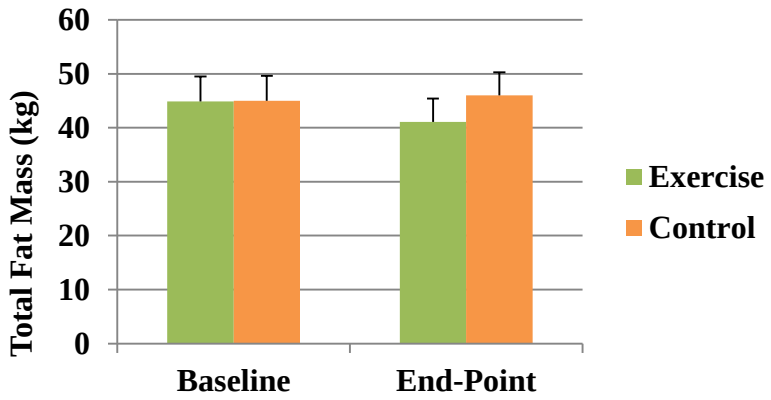
Figure 4.4 Estimated Marginal Means and Standard Deviation of %BF for the Exercise and Control Group at Baseline and End-Point Testing



The independent t-tests did not produce significant differences in mean FM between the exercise and control group at baseline testing, t(8) = -0.01, p=0.10 and end-point testing, t(8) = -0.81, p=0.44. Levene's Test of Equal Variance was not violated for the two independent t-tests run (p>0.05) (exercise vs. control group at baseline, exercise vs. control at end-point); therefore the Equal Variance Assumed values were used.

The pairwise comparisons for within group differences across the two testing time points produced no significant result for FM. However there was a trend towards significance for the exercise group in FM between baseline and endpoint testing, t(4) = 2.70, p=0.054.

Figure 4.5 Estimated Marginal Means and Standard Deviation of FM for the Exercise and Control Group at Baseline and End-Point Testing



4.2.3 Circulating Leptin

Raw leptin level data is displayed in Appendix R. Results of the multiple linear regression for circulating leptin levels at endpoint testing are displayed in Table 4.4. The independent variables initially included in the model were age intervention group status (exercise vs. control), and percent change in BMI, weight, %BF, FM and LM from baseline to endpoint testing.

The only variable found to be significant in predicting leptin levels at end-point testing was the percent change in %BF (t(8)=0.01, p<0.01). This indicated that %BF accounted for 58.9% of the variance in end-point leptin levels (R^2=0.589).

Table 4.4 Stepwise Multiple Linear Regression Analysis for End-Point Circulating Leptin Values

	B	SE B	β	R^2
Step 1				
%BF	6.915	2.041	0.768*	.589

*p<0.01
Abbreviation: Percent Body Fat (%BF)

Results for the 2x3 factorial ANCOVA for circulating leptin levels are presented in Table 4.5. Percent change in %BF from baseline to end-point testing was used as covariate as it was the only independent variable that was significant from the stepwise multiple linear regression for end-point leptin levels. Baseline leptin levels were also used as covariate in the ANCOVA. No violations were found for sphericity when consulting Mauchly's test of Sphericity $\chi^2(2) = 0.55$, p=0.91, therefore Sphericity Assumed results were consulted for within group differences.

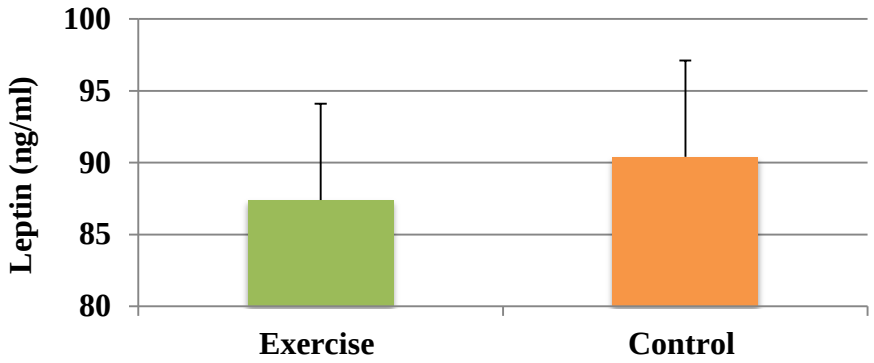
Table 4.5 Circulating Leptin Levels for the Exercise and Control Group at Baseline, Mid-Point and End-Point Testing

	Baseline Testing		**Mid-Point Testing**		**End-Point Testing**	
	Exercise Group	**Control Group**	**Exercise Group**	**Control Group**	**Exercise Group**	**Control Group**
Leptin (ng/ml)	82.4±23.6	109.9±35.2	59.0±20.0	106.6±22.9	73.1±32.2	102.2±27.0

Data are displayed as Mean ± SD
Comparison was made between groups (exercise vs. control) and over time (baseline vs. mid-point vs. endpoint) using a 2x3 factorial ANCOVA
Covariates: %BF & baseline leptin levels

A significant group main effect for leptin levels was found between the exercise and control group F(1,16.1), p=0.01 when controlling for baseline leptin levels and %BF. This indicates that when the time point variable is ignored, there is a significant change in circulating leptin levels between the two groups (exercise vs. control). However, a pairwise comparison between the exercise and control group was not significant (p=0.81). Figure 4.6 displays the marginal means for leptin levels for the exercise and control group.

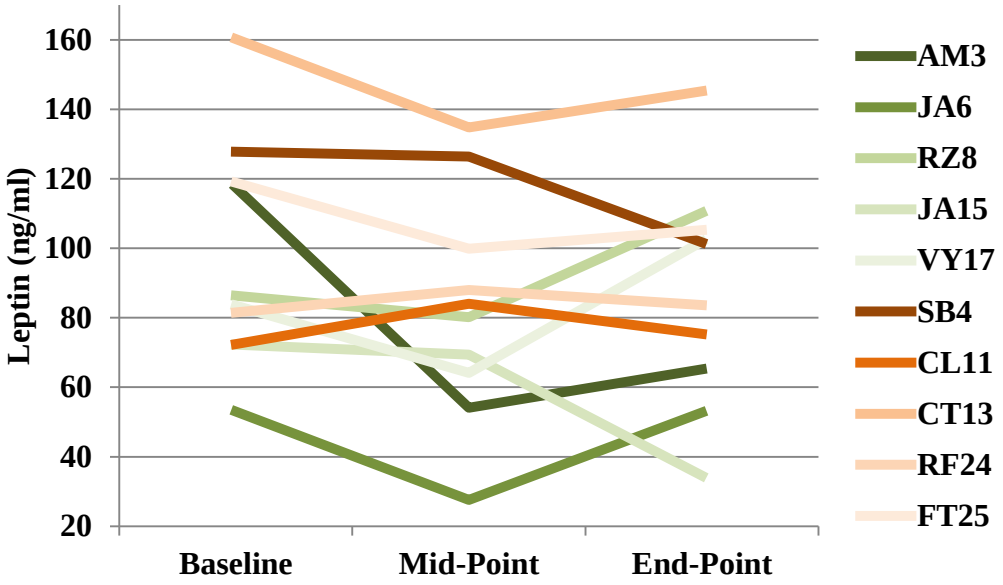
Figure 4.6 Estimated Marginal Means and Standard Error of the ANCOVA Group Main Effect for End-Point Circulating Leptin Levels



There was no interaction effect between group (exercise vs. control) and time point (baseline vs. mid-point vs. end-point) for leptin levels when controlling baseline leptin levels and %BF ($F(2,2.8)$, $p=0.98$,). Also there was no main effect of time for leptin levels when controlling for baseline leptin levels and %BF ($F(2,2.4)$, $p=0.13$).

When graphically represented, the individual leptin data (Figure 4.7) for participants in the exercise group showed a tendency to decrease from baseline to mid-point testing and then displayed an increase from mid-point to end-point testing. The control group showed a fairly consistent trend of increasing leptin level from baseline to mid-point testing. From mid-point to end-point testing, most control participant's leptin levels seem to either decrease slightly or plateau. It is noted however that there were two control participants who appeared to follow a similar trend compared to the exercise group (decreases in leptin levels), although the amount that their leptin levels decreased did not appear to be as prominent as the exercise group. This individual variation between the exercise and control group participants coupled with the small sample size may explain the lack of difference in the leptin levels between the exercise and control group.

Figure 4.7 Individual Participant Circulating Leptin Level for Baseline, Mid-Point and End-Point Testing
*Green lines represent exercise group & orange lines represent control group



4.2.4 Circulating KiSS

Raw KiSS level data is displayed in Appendix S. The stepwise multiple linear regression for circulating KiSS levels at endpoint testing showed no significance. The independent variables that were initially included in the model were age, intervention group status (exercise vs. control) and percent change in BMI, weight, %BF, FM, LM and circulating leptin levels from baseline to endpoint testing. None of the assessed independent variables had a significant contribution to endpoint circulating KiSS levels ($p>0.05$).

Results for the 2x3 factorial ANCOVA for circulating KiSS levels are display in Table 4.6. Since there were no independent variables that were significant predictors of end-point KiSS levels in regression analysis, baseline KiSS levels was the only covariate used. No violation were found for sphericity when consulting Mauchly's test of Sphericity $\chi^2(2) = 2.70$, $p=0.26$. There was a main effect of time $F(2,4.6)$, $(p=0.03)$, indicating that when the grouping variable (exercise vs. control) was ignored, there was a significant change in KiSS over the three testing time points (Figure 4.8). The Test of Within-Subjects Contrasts indicated that the significant difference was

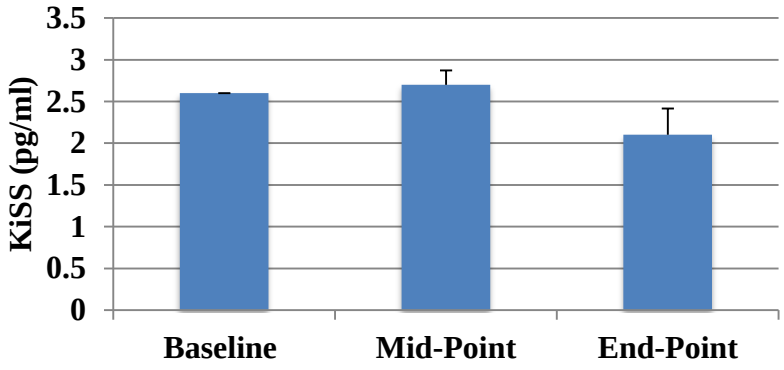
between baseline and end-point testing (p=0.05). Figure 4.9 displays the estimated marginal means and standard deviation for KiSS at baseline, mid-point and end-point testing. There were no main effects for group (exercise vs. control) F(2,0.046), p=0.10) or interaction between group (exercise vs. control) and time (baseline vs. mid-point vs. end-point) (P>0.05) for KiSS levels.

Table 4.6 Circulating KiSS Levels for the Exercise and Control Group at Baseline, Mid-Point and End-Point Testing

	Baseline Testing*		**Mid-Point Testing***		**End-Point Testing***	
Variables	**Exercise Group**	**Control Group**	**Exercise Group**	**Control Group**	**Exercise Group**	**Control Group**
KiSS (pg/ml)	2.6±0.7	2.6±0.9	2.8±0.5	2.7±0.5	2.1±0.7	2.1±1.1

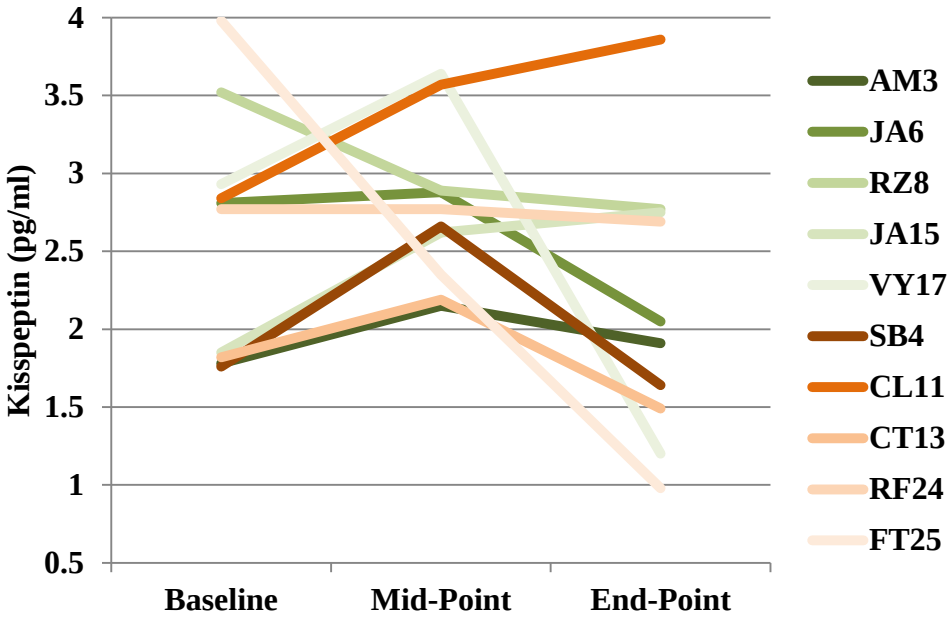
Data are displayed as Mean ± SD
Comparison was made between groups (exercise vs. control) and over time (baseline vs. mid-point vs. endpoint) using a 2x3 factorial ANCOVA
Covariates: Baseline KiSS levels
Abbreviation: Kisspeptin (KiSS)
*Significant Time Effect (P<0.05)

Figure 4.8 Estimated Marginal Means and Standard Error of the ANCOVA Time Main Effect for KiSS levels; Abbreviation: Kisspeptin (KiSS)



Unlike weight and circulating leptin levels, there does not appear to be a clear trend between the exercise and control group when individual participants' KiSS data is graphically represented (Figure 4.9). The majority of both of the exercise and control group participants displayed an increase in circulating KiSS from baseline to mid-point testing and then a decrease in KiSS levels from mid-point to end-point testing.

Figure 4.9 Individual Participant Circulating KiSS Level for Baseline, Mid-Point and End-Point Testing
*Green lines represent exercise group & orange lines represent control group.



5.0 DISCUSSION

The aim of this study was to determine if a 12-week exercise intervention for obese women would result in a significant decrease in FM and circulating leptin levels, and a significant increase in KiSS levels. The three main results were (a) a significant decrease in %BF from baseline to end point testing in the exercise group compared to the control group; (b) a significant main effect of group (exercise vs. control) on circulating leptin levels and (c) a significant main effect of testing time point (baseline vs. mid-point vs. end-point) on circulating KiSS levels.

5.1 Percent Body Fat, Fat Mass & Weight

Women in the exercise group experienced a significant decrease in %BF ($p<0.01$) (46.7±3.3% to 44.6±3.8%) and a trend towards a significant decrease in FM ($p=0.054$) (44.9±11.9kg to 41.1±10.8kg) from baseline to end-point testing; whereas the control group

maintained their %BF ($p>0.05$) (45.7±3.5% to 46.2±3.6%) and FM ($p<0.05$) (45.0±8.3kg to 46.0±8.3kg) between baseline and end-point testing. These findings are consistent with the stated hypothesis that women in the exercise group would experience a significant decrease in FM compared to the control group.

There was no significant effect of the exercise intervention on body weight or BMI in the exercise group. This failure to observe a change in these two variables, while at the same time observing a significant change in FM and %BF is often explained by an increase in LM as LM encompasses all other weight (other than FM) that makes up body mass (McArdle, Katch, Katch, 2010). However, there were no changes in LM in either the exercise or control group.

The findings in the present study are consistent with those observed by Arikawa et al. (2011) who also found a significant decrease in %BF and FM in obese women after a 16-week exercise intervention (45-minute sessions x 5-days a week), but failed to show changes in weight and BMI (Arikawa et al., 2011). It is important to note however, that LM was not measured. Polak et al. (2006) found that women who completed an exercise intervention (45-minutes, five times a week for 12-weeks) had a significant decrease in %BF. A study by Kondo et al. (2006) also found that obese women after completing an exercise intervention (30-minutes, four times a week for 28-weeks) showed a significant decrease %BF and FM. However unlike Arikawa et al. (2012) and the current study, significant decreases in weight and BMI were also observed in both these studies (Polak et al., 2006; Kondo et al., 2006). Two other studies, Sari et al. (2007) and Aziz (2012), also showed a significant decrease in BMI in obese women after an exercise intervention, changes in weight, %BF or FM were not monitored though. In Sari et al. (2007) the exercise intervention consisted of exercising for 45-minutes, five times a week for 4-weeks. Azizi (2012) on the other hand, had participants exercise for 60-minutes, three times a week for 8-weeks. The total volume of an exercise program can have a significant impact on body composition. However, the lack of difference in total exercise volume among the mentioned

studies makes it difficult to determine if it was a contributing factor to whether or not studies, including the current one, experienced a significant change in LM, weight or BMI.

In addition to the total volume of an exercise intervention, the mode of exercise used in interventions may also explain the lack of change in weight, BMI and LM despite the observed change in %BF and FM in the current study. In the current study only aerobic exercise was used as part of the intervention, whereas Kondo et al. (2007), who saw an increase in LM, used a combination of aerobic and resistant training exercises. Resistance training exercises are much more effective in increasing LM compared to aerobic exercise alone (McArdle et al., 2010). Therefore, the lack of resistance training in the current study may explain why changes in LM weight or BMI did not change.

Adherence to the exercise intervention is also an important parameter to consider when interpreting the findings of any exercise study. Exercise creates an energy deficit, which is needed to cause a change in body composition (McArdle et al., 2010). If adherence decreases during an exercise intervention, it may lead to a plateau in weight loss as a large enough energy deficit may no longer be created. Thus, maintaining or decreasing one's adherence to an exercise intervention might explain a change (or lack of) in anthropometric parameters.

In the current study the average adherence rate was 72.9% and ranged from 58.3 – 100%. Although this is considerably higher than typically observed (less than 50% - Robinson & Rogers, 1994), it is important to note that there was a large drop off in attendance from midpoint to end-point testing by three of the participants (JA6, RZ8, VY17) in the exercise group. For example, participant JA6 experienced a decrease in weight from baseline to mid-point and then an increase in weight from mid to end-point testing. When her exercise attendance record was reviewed it showed that she attended fifteen out of the maximum sixteen exercise sessions during the first four weeks of the exercise program, however she only attended three out of sixteen exercise sessions in the last four weeks of the program. It was also noted that during the last four

weeks she missed exercise sessions due to illness and work commitments. Participants RZ8 and VY17 also presented with similar trends in their body weight over the intervention. This drop off in attendance by each of these participants would have likely lead to less of an energy deficit, which would prevent further weight loss or even caused weight gain. This may help to explain why there was no significant difference in weight, LM and BMI between the exercise and control group at end-point testing (Figure 4.2).

There are numerous methods that can be used to measure and/or estimate body composition. When comparing the methods used to measure body composition in the current study to other exercise intervention studies there is a lack of consistency across the literature that may explain the conflicting results. Two common methods that are used in studies are DXA or bioelectrical impedance (BIA) (Neiman, 2011).

The current study used DXA to measure body composition and found a significant decrease in %BF and a trend towards significance in FM, but no change in LM. The only other study to report significant LM findings was Kondo et al., (2006), who used BIA to measure body composition. BIA is a non-invasive tool used to measure body composition, and can become unreliable when measuring at the extreme ends of body composition (McArdle et al., 2010), such as might be observed in obese populations. When comparing the use of DXA to BIA in obese males, it was determined that BIA significantly over predicted LM and under predicted FM (Pateyjohns et al., 2006). Therefore the results found by Kondo et al., (2006), which included a significant decrease in %BF, FM and LM, may be skewed. This suggests that like the current study, LM may not have changed in Kondo et al, (2007) despite possible changes in %BF and FM.

The underpowered sample size and the large variability in data across the participant pool may also explain the failure to observe a significant change in weight, LM and/or BMI. An underpowered sample size is more likely to cause a type II error, meaning that a significant

difference in LM, weight or BMI may have occurred, but was not statistically detected (Jones et al., 2003). The current study was underpowered to detect changes in leptin, and thus may have been similarly underpowered to observe changes in BMI, body weight and/or LM. Studies such as Polak et al. (2006), where a significant decrease in both body weight and BMI was found, had a sample size of twenty-five. Also studies by Aziz (2012) and Sari et al. (2008), who both observed a significant decrease in BMI, had total sample sizes of twenty-four and twenty-three participants respectively. This is in contrast to the current study that had a total sample of only ten participants (across two groups).

Overall, there was a significant decrease in %BF and almost significant decrease in FM in the exercise group however weight and BMI remained unchanged. There was no significant increase inLM among the exercise participants, therefore it cannot be used to explain the lack of change in weight and BMI. It is more likely that the underpowered sample size and large variability among participants that caused the inconsistent results.

5.2 Circulating Leptin

The 2x3 factorial ANCOVA found no group x time interaction for circulating leptin levels. However a significant group main effect was noted ($p<0.01$). This suggests that regardless of the testing time point leptin levels were significantly different between the exercise and control group. Overall, these results do not support the hypothesis that the exercise group would experience a significant decrease in circulating leptin levels compared to the control group.

The literature remains unclear with respect to the effect of an exercise intervention on circulating leptin levels in obese women. Some studies have demonstrated a significant decrease in leptin levels after an exercise intervention, whereas others have failed to demonstrate a change. Findings of Arikawa et al. (2011) are similar to those of the current study where there was no significant change in leptin levels after an aerobic exercise intervention (45-minutes, 5-

days/week for 16 weeks) for obese women. Kramer et al. (1999) also failed to demonstrate changes in leptin levels in obese women participants after a 9-week aerobic exercise intervention (30-minutes 4-days/week). Interestingly, Volpe et al. (2008) found that obese women randomized to the exercise only group (30-minutes, five times a week) did not demonstrate a decrease in leptin, whereas those in the diet or a diet and exercise group saw a significant decrease in circulating leptin levels after a 26-week intervention.

Although there are numerous studies that have not shown a decrease in circulating leptin levels in obese women after an exercise intervention there are a number of studies that have. Polak et al. (2006) demonstrated a significant decrease in circulating leptin levels in obese women after a 12-week aerobic exercise intervention (45-minutes, 5-days/week). Similar to Polak et al. (2006), Sari et al. (2007) and Azizi (2012) both showed significant decreases in leptin levels in obese women after aerobic exercise interventions. Sari et al. (2007) had participants exercise for 45-minutes, 5-days a week for 4-weeks, whereas Azizi (2012) used a protocol that involved exercising for 60-minutes three times a week for 8-weeks. Finally, Kondo et al. (2006) used aerobic and resistance training in combination and found that over a 28-week exercise intervention (30-minutes, 3/4 times a week) obese women had a significant decrease in leptin levels.

It has been suggested that a decrease in adipose tissue such as that measured by %BF or FM needs to be attained before leptin levels decrease since leptin is produced and released from FM (Lee & Fried, 2009; Matsubara et al., 2002, Kraemer et al., 1999). For example participants in Kondo et al. (2007) experienced a 14% decrease in %BF as well as a significant decrease in leptin. Also in Polak et al. (2006), participants demonstrated roughly a 6.0% decrease in %BF and showed a significant decrease in leptin levels. This evidence would suggest that participants in Polak et al. (2006) experienced a large enough reduction in adipose tissue to see a decrease in leptin levels. While Sari et al. (2007) and Azizi (2012) also showed a significant decrease in

leptin only BMI was used as the body composition measurement and thus cannot be directly compared.

When compared to studies that did not experience a change in leptin levels there was a smaller magnitude of change in %BF or no change at all. For example, in the current study where no change in leptin was found, there was only a 4.57% decrease in %BF over the intervention. Arikawa et al. (2011), who also did not see a significant decrease in leptin, experienced only a very small magnitude of change in %BF (-0.94%). Kraemer et al. (1999) and Volpe et al. (2007) both failed to show any change in %BF and circulating leptin levels.

Although there was a significant change in %BF in the current study, it is suggested that the magnitude of change was not large enough to lead to a decrease in leptin. Kraemer et al. (1999) speculated that the large FM and elevated leptin levels in obese individuals necessitates a larger decrease in %BF or FM to cause a decrease in leptin levels due to the leptin resistance experienced by these individuals. Similarly, Volpe et al. (2008) speculated that the lack of change in FM was a contributing factor as to why circulating leptin levels did not decrease in the exercise only participants.

Another factor that may have contributed to the discrepancy in leptin change across the various exercise interventions may be the actual amount of exercise performed in each study. The American College of Sports Medicine (ACSM, 2010) recommends an energy expenditure of $\geq$ 2000kcals a week, which translates to 250 to 300-minutes a week of exercise for an intervention to cause a large enough energy deficit to loose or maintain weight. Individuals who expend less that 2000kcals, but more than 1000kcals or 150-minutes of exercise a week will still gain a minimum number of health benefits from exercise, however weight loss or maintenance of weight may not be achieved at this exercise volume (ASCM, 2010).

The current study did not meet ACSM's recommendations for weight loss (220-minutes/week), a factor that might also help to explain the failure to observe a significant change

in body weight and leptin levels. Similarly, Arikawa et al. (2011) who also failed to meet ACSM's guidelines (225-minutes/week) did not see a significant decrease in weight or leptin levels, even though %BF decreased. Kraemer et al. (1999) and Volpe et al. (2008), who both failed to show decreases in weight, FM, %BF and leptin levels, also did not meet ACSM's guidelines for weight loss or maintenance. Participants in Kraemer et al. (1999) exercised a maximum of 120-minutes a week whereas participants in Volpe et al. (2008) only exercised 150-minutes a week.

In Kondo et al. (2006), where a significant decrease in weight, %BF and leptin was experienced, the exercise intervention was tailored to promote a 400 – 500kcal deficit each session. This meant that participants met the ACSM recommendations as 2000kcals per participant were expended a week (28 weeks x 4 sessions a week). Polak et al. (2006) had participants exercise for 45-minutes, five times a week for 12-weeks, which also met the recommended amount of time (300-minutes). They too demonstrated a decreased in weight, %BF and leptin levels.

The exercise interventions used in Sari et al. (2007) and Azizi (2012) were both slightly under the recommended time per week to achieve weight loss interestingly however, participants in both studies did experience significant decreases in BMI and leptin levels (Sari et al. 2007; Azizi, 2012). This would suggest that despite not meeting the ACSM (2010) recommendations there was till a large enough energy deficit to cause a decrease in leptin levels.

Although there were no significant changes in weight, BMI or LM for the exercise group in the current study, it is interesting to note that when the relationship between individual participant leptin data (Figure 4.2.7) and weight data (Figure 4.2.2) was compared trends did emerge. Specifically, as weight decreased in individual participants in the exercise group from baseline to mid-point testing so did leptin levels, suggesting that enough energy had been expended to cause a decrease in leptin. From mid to end-point testing the trend was reversed as

three out of five exercise participants (JA6, RZ8, VY17) experienced an increase in weight and leptin levels. These data further support the premise that changes in body weight elicit responsive changes in leptin levels.

The control group also expressed trends between weight and leptin. From baseline to mid-point testing all control participants experienced a significant increase in weight with a corresponding increase in circulating leptin in three of the five participants.

A final factor that cannot be ignored was the small sample used in the current study. It was calculated that a minimum of sixteen participants total were needed to complete the study to ensure it was powered to detect any changes in leptin levels. However only ten participants completed the study, which suggests that leptin levels may have significantly changed, but due to the study being underpowered it was not statically detected. Further studies with a larger sample sizes are suggested to confirm the potential trends demonstrated between the leptin and weight data.

5.3 Circulating KiSS

No variables, including circulating leptin levels were found to significantly predict changes in end-point testing KiSS level. Similarly, there was no group x time interaction found for circulating KiSS. However, there was a main effect for time suggesting that KiSS changed similarly over the three testing time points across the two groups. Taken together these results do not support the hypothesis that the exercise group would experience an increase in circulating KiSS compared to the control group over the exercise intervention.

There have been a limited number of previous studies that have examined the relationship of KiSS to leptin. Leptin receptors have been located on KiSS neurons in the hypothalamus of ewes, mice and humans (Backholer et al., 2010; Smith et al., 2006). As well, studies have demonstrated that leptin may control KiSS production. For instance, a lack of leptin has been

shown to lead to decreases in KiSS mRNA (Luque et al., 2007; Quennell et al., 2011), whereas exogenous injections of leptin increased KiSS mRNA production (Luque et al., 2007; Morelli et al., 2008; Backholer et al., 2010).

It was the premise of the current study that if exercise was able to decrease leptin levels in obese women an increase in KiSS production would follow. It has been shown that obese rodents who have extremely high circulating leptin levels, display a down regulation in KiSS production (Quennell et al., 2011) and that the fall in KiSS production may be a consequence of 'leptin resistance'. Leptin resistance is a decrease in the sensitivity of leptin to its OB-Rb or the inability of leptin to reach its OB-Rb. In obese individuals it has been suggested that elevated leptin levels lead to leptin resistance (Myers et al., 2012). By "reversing" this leptin resistance via reducing FM and then subsequently decreasing leptin levels, it is proposed that the sensitivity of leptin to its OB-Rb would be enhanced, or leptin's ability to reach its receptors would be improved (Myers et al., 2012). The subsequent premise being that the overall improved leptin signaling would restore KiSS production and lead to an elevation in circulating KiSS.

Although the current premise that a decrease in circulating leptin would improve leptin signaling and lead to an increase in KiSS production was not found, it is of interest to note that the hypothesized trends for weight, leptin and KiSS in the exercise group did present when individual data was examined. Specifically, four out of the five exercise participants (AM3, JA6, JA15, VY17) who experienced a decrease in weight and leptin levels from baseline to mid-point testing showed an increase in KiSS levels during the same time frame. However, these same four exercise participants (AM3, JA6, RZ8, VY17) had an increase weight and an increase in circulating leptin and a decrease in KiSS levels from mid to end-point testing. Only one exercise participant continued to see a decrease in weight and leptin levels from mid to end-point testing and a continued increase in KiSS levels during the same time frame. Thus, while this only represents select individual data, it does support the proposed premise that a reduction in leptin

resistance via reduced leptin levels may partially restore leptin signaling and cause an increase in circulating KiSS.

Interestingly this expected relationship between weight, leptin and KiSS does not hold when one examines the data across individuals in the control group. The small sample size and large variability amongst the data undoubtedly had a significant impact on the nature of the trends observed in the control group. It is however also important to note the timing of data collection and the timing of the menstrual cycle in which a number of control participants were tested did not take place within the prescribed protocol. It is well known that E_2 decreases KiSS production (Franceschini et al., 2006; Gottsch et al., 2009; Quennell et al., 2011). Thus, it was important that participants were tested between days six and nine (mid follicular phase) of the menstrual cycle when E_2 is lowest (Hall, 2009). There were a number of control participants however who were unable to be tested during the six to nine day window due to illness or other commitments. Thus, the measures of circulating KiSS from these participants reflect values influenced by different circulating E_2 levels. For example, end-point testing for participant FT25 was preformed on day 15 of her menstrual cycle, a time when E_2 levels would have been elevated (compared to day 6-9). E_2 levels may therefore explain the decrease in KiSS that was observed in this participant, even though there was a decrease in her leptin levels at the same time point.

Time of testing during the phase of the menstrual cycle may also explain the increase in KiSS, despite the increase in weight and/or leptin levels that was observed in two control participants during mid-point testing. Just prior to ovulation on day 14, LH and E_2 levels increase as E_2 switches from a negative feedback to a positive feedback system (Hall, 2009). Research has suggested that KiSS may mediate the positive E_2 feedback as E_2 injections during pre-ovulation increased KiSS mRNA (Dhillo et al., 2007). Specifically, Dhillo et al. (2007) demonstrated that when women were in injected with KiSS during different phases of the menstrual cycle, KiSS

had the greatest effect on increasing LH during the pre-ovulatory phase (when E_2 also increases). When looked at in relation to the current study this may help to explain why participants SB4 and CT13 saw increases in KiSS during mid-point testing even when leptin levels had increased as they had their mid-point testing done during days 11 and 12, which is close to ovulation (day 14), respectively.

This study demonstrated that this exercise intervention was not successful in increasing KiSS levels. A lack of significant change in leptin levels as well as the failure to control the time of the menstrual cycle during testing may explain the lack of significant change observed KiSS levels. Trends between the weight, leptin and KiSS data suggest KiSS levels may have increased in the exercise group as weight and leptin levels decreased.

5.4 Limitations

The major limitation of this study was the small sample size, which caused the study to be underpowered. It was calculated that the minimum number of participants needed to detect changes in leptin levels over the intervention was sixteen; indicating eight participants were needed for each of the exercise and control group. However, only ten participants in total completed the study. An underpowered study is more likely to produce a type two error, meaning the null hypothesis is accepted (there is no difference between two groups); when in actuality the alternative hypothesis is true (there is a difference between two groups) (Jones, Carley, Harrison, 2003). It is possible that the exercise intervention did have a positive impact on circulating leptin (experience a decrease) however the variability amongst such a small number of participants did not elicit a significant difference between groups.

Another limitation was the inability to collect blood samples across the full participant group within the same time period of the menstrual cycle. A number of participants in the control group were unable to be tested between day six and nine of their menstrual cycle due to illness or

work commitments. This meant that hormones such as E_2 might have affected the level of circulating KiSS, as E_2 is known to down regulate KiSS production (Smith et al., 2006). Thus this may have resulted in lower circulating KiSS levels for some participants. Also a number of the participants failed to hand in their exercise tracking sheets (APPENDIX A & C) at the end of study, which may have prevented an accurate calculation of adherence to the exercise intervention. This may have given a false impression of the total amount of energy deficit experienced by participants due to the intervention. Finally, participants in both the exercise and control group did not followed the dietary instructions leading up to the mid and endpoint testing sessions. Only two participants (one each in the exercise [AM3] and control group [SB4]) followed the same dietary intake the 24-hours preceding mid and end-point testing that they recorded for the 24-hour prior to baseline testing. Participants were asked to follow the same dietary regime for mid and end-point testing as they did baseline testing because acute nutritional intake can affect acute circulating leptin levels (Lee & Fried, 2008). Thus leptin levels obtained at mid and end-point testing may not be a true representation as participant's dietary intake 24-hours prior to testing could have acutely increased or decreased leptin levels.

5.5 Strengths

The primary strength of this pilot study is the observations that have been noted for KiSS. There are still very few studies that have assessed circulating levels of KiSS in obese women. Much of this research is still performed in animal models. The study also offers some contribution to the investigation of the relationship between obesity, metabolism and reproduction, as it is the first study to the author's knowledge that looks at the changes in circulating KiSS and leptin after an exercise intervention in obese women.

Another strength of the study was the inclusion of a control group. The use of a control group enabled the true effect of the exercise intervention on FM, circulating leptin and KiSS

levels to be ascertained. Without a control group, one would not be able to determine if it was the exercise intervention itself or an unknown variable that was responsible for the changes observed in %BF and FM in the exercise group.

A final strength was the supervised nature of the exercise intervention. This likely led to increased compliance to the exercise intervention which itself was an important strength. Anecdotal evidence from participants in the exercise group, suggested that being supervised made them more accountable to performing the exercise intervention. This meant that participants experienced greater benefits from exercise, such as FM loss. Previous research in the area of adherence and compliance to exercise also supports this notion. Cox et al., (2003) found that women who were supervised had great adherence to a 6-month intervention compared to those who were unsupervised.

5.6 Future Directions

Since this was a pilot study and is the first study to the author's knowledge to investigate the changes in KiSS after an exercise intervention, further studies that include a fully powered sample size, more controlled methods to ensure testing takes place during the proper time in the menstrual cycle and adherence to dietary intake prior to testing are suggested. Also, further studies that measure changes in fertility in an obese infertile women population are needed as the current study recruited obese women who displayed normal menstrual cycles.

Studies investigating the effects of an exercise intervention on pubertal development in obese and non-obese women adolescents should also be considered. KiSS not only controls the menstrual cycle but is also an important hormone involved in puberty. Rodents, mice and humans who possess mutations in the KiSS gene or the KiSS-R gene display a lack of pubertal development (Seminara et al., 2003; d'Anglemmont de Tassigny et al., 2007). Some research suggests that increased obesity among pre-pubescent women may alter the timing of puberty

(Biro, Greenspan, Galvez, 2012). Therefore, it is critical to study the relationship between obesity, puberty and exercise in this population as well.

Lastly, specific changes or modifications to the exercise intervention should be studied in order to maximize the potential benefits of the exercise intervention. There are three main suggestions to improve the current intervention; (1) more variability in the exercise program, (2) reduced caloric intake, and (3) strategies to maintain motivation to exercise. Although the aerobic exercise program did employ the use of the overload principle (increased time on the treadmill), the addition of resistance training may provide further benefits. Research has suggested that resistance training in combination with aerobic exercise is far better than aerobic exercise alone to induce weight and FM loss (ACSM, 2009). This would be beneficial for obese women as literature suggests that a large decrease in available energy is needed to experience decreases in circulating leptin (Hilton & Loucks, 2000) and thus proposed increases in KiSS.

There was no caloric restriction as part of the current study, as the main hypothesis was to study the effect of exercise. However, exercise and caloric restriction are often used together to decrease FM, as it focuses on the amount of energy expended (exercise) and the amount of energy that is taken in (diet). A Cochrane review by Shaw, Gennat, O'Rourke, Delmar (2009) found that when both exercise and diet were modified (increased caloric restriction) individuals were more successful in achieving weight loss compared to exercise alone. Lakhdar et al., (2013) also found that obese women who were randomized to a diet or diet and exercise group lost significantly more FM compared to the exercise only group. These two studies demonstrate the importance of caloric restriction through diet to decrease FM, which in turn may decrease circulating leptin levels and potentially increase KiSS levels. .

When reviewing the amount of exercise sessions attended by the exercise group, there was a large drop off in attendance from mid-point to end-point testing by three of participants.

The use of exercise psychology techniques and incentives to help prevent relapse in exercise behavior may help to attenuate this loss (Weinberg & Gould, 2007).

5.7 Conclusion

The current research project is the first known study to investigate changes in both circulating KiSS and leptin levels in obese women as a function of a 12-week exercise intervention. Although the study did not find a significant group x time interaction for circulating KiSS or leptin levels, a significant decrease in %BF and FM was found in the exercise group when compared to the control group.

The lack of significant decreases in circulating leptin levels in the exercise group may be due the small sample size and large variability in the date between the exercise and control group. Also the magnitude of change in %BF and FM levels in the exercise group may not have been large enough to lead to a decrease in leptin levels. It is suggested that circulating KiSS levels did not increase in the exercise group because there was not a significant decrease in circulating leptin and potentially due to the menstrual phases that some of the control group participants were in during mid and end-point testing. Overall it is recommended that studies with larger sample sizes and enhanced controlling of testing time points of participants and dietary tracking adherence are needed.

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APPENDIX A: Alternate Location – Training Log

Training Log – Alternative Location for 4th Exercise Session per Week

Date & Location	Treadmill Yes/No	WU Time	WU Heart Rate	WU Treadmill Speed	ME Time	ME Heart Rate	ME Treadmill Speed	CD Time	CD Heart Rate	CD Treadmill Speed
Example Feb 12/13 home gym	*Yes*	*10 minutes*	*10 x 6 =60 beats per minute*	*3 miles/hour*	*30 minutes*	*120 beats per minute*	*3.5 miles/hours*	*10 minutes*	*98 beats per minute*	*3 miles/hour*
Example Feb 15/13 walk outside	*No*	*10 minutes*	*10 x 6 =60 beats per minute*	*N/A*	*30 minutes*	*125 beats per minute*	*N/A*	*10 minutes*	*97 beats per minute*	*N/A*

APPENDIX B: Gym Training Log

TRAINING LOG

DATE	WU Time	WU Heart Rate	WU Treadmill Speed	ME Time	ME Heart Rate	ME Treadmill Speed	CD Time	CD Heart Rate	CD Treadmill Speed
Example March 12, 2013	*10 minutes*	*100 beats per minute*	*3 miles/hour*	*30 minutes*	*120 beats per minute*	*3.5 miles/hours*	*10 minutes*	*98 beats per minute*	*3 miles/hour*

APPENDIX C: Team 2 Polar System

POLAR TEAM² QUICK GUIDE

This is Quick guide for the Polar Team². Please read Quick guide through before beginning to work with Team². Quick guide gives you the basic information on Team². For more information, see Team² software help.

POLAR TEAM² INCLUDES

1 POLAR TEAM² BASE STATION

- Connects the transmitters with your PC
- Allows you to monitor up to 80 players in real time
- Ethernet, Wi-Fi, Bluetooth
- Rechargeable battery with up to 12 hours stand-alone use
- Operating temperature -20°C - +60°C (-4°F - +140°F)
- Water-resistant if the plug is in place

NOTE: During charging the legs of the base station must be used because the bottom heats up. The bottom of the base station heats up to 60°C (140°F). During charging, the maximum operating temperature is 45°C (113°F).

2 10 POLAR TEAM² TRANSMITTERS AND TRANSMITTER STRAPS

- Transmitter compatible with most Polar wrist units
- Transmitter strap exclusively designed for Team² transmitters
- Transmitter memory can record 48 hours of data in online mode and up to 360 hours of data in off-season mode
- Bluetooth, Polar magnetic communication technology transmitter, re-chargeable battery
- Water-resistant

3 POLAR TEAM² CHARGER

- Not water-resistant, only for indoor use

USB DONGLE

- Secondary option for downloading data from the transmitters' memory
- Bluetooth

POLAR TEAM² PC SOFTWARE

- Installation instructions included in Quick guide
- Download the latest software updates to get the newest features for your Team²

POLAR
LISTENS TO YOUR BODY

APPENDIX D: Outside Study Protocol Physical Activity & Exercise Log

Physical Activity & Exercise Journal

- This journal is to record any physical activity or exercise that is **done outside of the study protocol**
- Please record the frequency, intensity, time and type of physical activity or exercise performed

Date (Frequency)	**Intensity**	**Time**	**Type**
Example *March 20, 2013*	*Light*	*30 minutes*	*Walking*

APPENDIX E: Recruitment Poster

College of Kinesiology
University of Saskatchewan

PARTICIPANTS NEEDED FOR RESEARCH ON CHANGES IN ADIPOCYTOKINES INVOLVED IN REPRODUCTION AND METABOLISM AFTER AN EXRCISE INTERVENTION

We are looking for **female** volunteers between the **ages of 18 and 45** with a body mass index (BMI)* of 30 or greater, who have a **regular menstrual** cycle, are **not pre-diabetic or diabetic** (type I or II) and are **not using hormone contraception** to take part in this study.

Study Purpose – To determine the effects of a 12-week exercise intervention on circulating metabolic and reproductive hormone levels in females with a BMI equal or greater than 30.

As a participant in this study, you will be randomized into one of two groups: a **12-week exercise group** or a **12-week non-exercise control group**

All participants will take part in **pre-screening** and **3 testing sessions** (pre-mid-end intervention)

- **Prescreening** (Estimated Time: 90 minutes)
    - BMI Calculation, Physical Activity Readiness Questionnaire, Physical Activity Questionnaire (PAQ), Self Reported Menstrual Cycle/Medical Disorders, Hemoglobin A1C Test
- **Testing Session** (Estimated time per session: 90 minutes)
    - 24-hour Dietary Log, Blood Draw, Submaximal Aerobic Exercise Test, PAQ, 3-Day Dietary Recall, Body Composition Assessment

If randomized into the exercise program your participation would include:

- Supervised exercise on a bicycle **3x week for 12-weeks** at the R.J.D. Williams Building
- Total time spent on the bike will start at **50 minutes** and increase **by 5 minutes every 2 weeks to a total of 75 minutes**
- Total time spent on study responsibilities compared to control group is **37.5 additional hours**

Benefits of participating in this study include finding out about your body composition, aerobic fitness level and supervised exercise sessions at no charge.

If you are **interested** and believe you are **eligible** please email Brittany Gadzosa at **bcg625@mail.usask.ca**
Primary Investigator – Dr. Carol Rodgers, College of Kinesiology

APPENDIX F: Power Calculation

Power Calculation

Method used to calculate sample size was taken from:
Jones, S.R., Carley, S., Harrison, M. (2003). An introduction to power and sample size estimation. British Medical Journal, 20, 453-458.

- The sample size was determined by averaging the calculated sample sizes from six different studies
- To calculate sample size from each study the following equation was used
    - Equation: Change in leptin levels/average standard deviation (SD) in leptin levels
        - Clinical Difference: Change in leptin levels (Starting point levels - Endpoint levels) seen in studies listed
        - Standard Deviation (SD): Calculated by averaging SD in leptin level experienced in studies listed

1) Aziz, M. (2012). Serum leptin and ghrelin changes-induced aerobic training in health young women. *International Journal of Collaborative Research on Internal Medicine & Public Health, 4*(6), 1257-1264.
   Change in leptin: 25.68 – 13.95 = 11.73
   SD: 2.9
   11.73/2.9= 4.0
   Suggested sample size at 80% power = 8

2) Kondo, T., Kobayshi, I., Murakami, M. (2006). Effects of Exercise on Circulating Adipokine Levels in Obese Young Women. *Endocrine Journal, 53*(2), 189-195.
   Change in leptin: 16.4 – 12.3 = 4.1
   SD: 4.6+5.4/2 = 5
   4.1/5 = 0.89
   Suggested sample size at 80% power = 26

3) Polak, J., Klimcakova, E., Moro, C., Viguerie, N., Berlan, M., Hejnova, J., Richterova, B., Kraus, I., Langin, D., Stich, V. (2006). Effect of Aerobic training on plasma levels and subcutaneous abdominal adipose tissue gene expression of adiponectin, leptin, interleukin 6, and tumor necrosis factor alpha in obese women. *Metabolism Clinical and Experimental, 55*, 1375-1381.
   Change in leptin: 24.3 – 18.7 = 6.2
   SD: 8.7+8.3/2=8.5
   6.2/8.5 = 0.7
   Suggested sample size at 80% power = 33

4) Kelly, K.,Blaszczack, A. Huas, J.M., Patrick-Melin, A., Fealy, C.E., Soloman, T.P., Kalinski, M.I., Kirwan, J.P. (2012). A7-day exercise program increases high-molexular weight adiponectin in obese adults. *Medicine and Science in Sport and Exercise, 44*(1), 69-74.
   Change in leptin levels:36.8 – 31.1 = 5.7
   SD: 5.1+4.2/2=4.7
   5.7/4.7 = 1.2
   80% power = 12

Average sample size between studies
8+21+33+12/4 = 18.5

APPENDIX G: Consent Form

PARTICIPANT INFORMATION AND CONSENT FORM

STUDY TITLE: Effects of a 12-week Exercise Intervention on Circulating Adipokines, Metabolic and Reproductive Hormone Levels in Obese Women

PRINCIPAL INVESTIGATOR:
Carol Rodgers Ph.D., Dean College of Kinesiology, University of Saskatchewan: 306-966-1061, carol.rodgers@usask.ca

SUB-INVESTIGATORS and STUDENT RESEARCHER:
Adam Baxter-Jones, M.S., Ph.D., Professor, College of Kinesiology, University of Saskatchewan: 306-966-5759, baxter.jones@usask.ca

Roger Pierson, M.S., Ph.D., FEAS, FCAHS, Professor, Department of Obstetrics, Gynecology and Reproductive Sciences, College of Medicine, University of Saskatchewan: 306-966-4058, pierson@erato.usask.ca

Patricia Doyle-Baker, M.S., DrPH, Associate Professor, Faculty of Kinesiology, University of Calgary: 403-220-7034, pdoyleba@ucalgary.ca

Brittany Gadzosa, B.Kin, Student Researcher, University of Saskatchewan: 306-966-1305/ 306-715-9643, bcg625@mail.usask.ca

24-HOUR CONTACT NUMBER: 306-715-9643 or bcg625@mail.usask.ca

INTRODUCTION
You are invited to take part in this research study because you are a women between the age of 18 and 45, menstruating regularly and, based on your body mass index (BMI), you are categorized as being above a healthy weight. BMI is a calculation that involves dividing your weight (kg) by your height $(m)^2$ and is used to determine if an individuals falls within a healthy weight category.

Your participation is voluntary. It is up to you to decide whether or not you wish to take part. If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

If you do not wish to participate, you will not lose the benefit of any medical care, employment, or academic standing, as applicable to which you are entitled or are presently receiving.

Please take time to read the following information carefully. You can ask the researchers to explain any words or information that you do not clearly understand. You may ask as many questions as you need. Please feel free to discuss this with your family, friends or family physician before you decide. If you would like your family doctor notified of your participation

in this study, the researchers will send a letter on your behalf to your doctors' office detailing the study protocol and testing procedures (Please see the end of this document if you would like your doctor notified).

WHO IS CONDUCTING THE STUDY?

The primary investigator of this study is Dr. Carol Rodgers from the College of Kinesiology and the sub investigators are Dr. Adam Baxter-Jones, Dr. Roger Pierson and Dr. Patricia Doyle-Baker. Brittany Gadzosa is the student researcher in the study and will have the majority of contact and interaction with you as a participant in the study.

WHY IS THIS STUDY BEING DONE?

This study is being done to investigate the effect of an exercise program on several hormones that affect women metabolism and reproduction. The primary hormones of interest are leptin and kisspeptin. Leptin and kisspeptin are both altered in above healthy weight states; leptin has been shown to increase from normal levels whereas kisspeptin has been shown to decrease from normal levels. The change in leptin and kisspeptin in increased weight states may be tied to infertility.

Weight loss through chronic exercise has been shown to reduce leptin levels in women above a healthy weight; however, there has been no research looking at the change in kisspeptin levels after an exercise program. This study is the first to examine the relationship between exercise, weight loss, leptin and kisspeptin.

WHO CAN PARTICIPATE IN THE STUDY?

You are eligible to participate in this study if you are a women between the age of 18 and 45, your BMI is $\geq 30 kg/m^2$, you are not currently pregnant, you have been weight stable and fairly inactive for the past 2-months and you have regular menstrual periods. Also, within the last 2-months you should not have been taking any form of hormone contraception or hormone replacement therapy. Finally, you should be free of any reproductive disorders such as Polycystic Ovary Syndrome or irregular menstrual cycles and free of other medical conditions such as insulin resistance or diabetes (Type I or II).

WHAT DOES THE STUDY INVOLVE?

This study has two phases: Phase I is screening to confirm eligibility for this study, and Phase II is the exercise intervention.

Phase I: Screening Procedures

To confirm your eligibility to safely participate in this study, Phase I will cover specific screening procedures to ensure you meet all inclusion criteria. These measurements will take place at the Physical Activity Complex (PAC) at the University of Saskatchewan campus (87 Campus Drive, Saskatoon, SK) and Royal University Hospital (103 Hospital Drive, Saskatoon, SK), be conducted by a combination of researchers and trained technicians, and take about 90 minutes. The screening measurements will include the following physical measures and questionnaires:

- Height and weight to calculate BMI
- Physical Activity Readiness Questionnaire for Everyone (PAR-Q+) to ensure you are cleared for unrestricted exercise (Note: If the PAR-Q+ does indicate a possible risk, you will be asked to have your doctor fill out and sign a Physician Physical Activity

Readiness Clearance form, indicating you are cleared to participate in unrestricted exercise)

- A 2-Month Physical Activity Questionnaire for adults (2-Month PAQ-AD) to determine your physical activity/exercise behavior over the prior two months
- Information about your reproductive history (frequency of menstrual cycles, and reproductive disorders) and any known medical conditions or diagnoses
- Hemoglobin A1c (HbA1c), a simple test that indirectly reflects your blood glucose levels over the past 3-months**.** After you have completed and met the screening criteria above you will be given a requisition form to have your HbA1c test done at the Royal University Hospital. The test involves a registered nurse or phlebotomist taking 7ml (1teaspoon) of blood from a vein at the inside of your elbow by a needle.
- Once the result of the HbA1c test is ready, Brittany Gadzosa will be responsible for picking up the official results from the Royal University Hospital. Brittany Gadzosa will then notify you of your HbA1c level, and based on the result, you will proceed to Phase II of the study or be notified that your HbA1c level does not meet the inclusion criteria and will not continue any further in the study.

Phase II: Exercise Intervention & Additional Study Testing Procedures

If you are fully eligible for this study (meeting all screening criteria listed above), you will be 'randomized,' or put into one of two groups by chance (like tossing a coin). One group is the non-exercise group, where you will be asked to maintain your current lifestyle behavior. Specifically, this means you will not start the exercise program offered in the study. If, on your own, you start to participate in physical activity or exercise, we will ask you to advise the study personnel about this so that you can be given a journal in which you will be asked to record the frequency, intensity, time and type of exercise or physical activity you perform. This journal will then be handed into the researchers at the completion of the study. As a member of the non-exercise group you will be provided with a 12-week pass to the PAC that will allow you to use the fitness equipment or take part in instructed exercise classes upon completion of the study.

If you are randomized into the exercise group you will participate in a 12-week exercise program. The exercise program will consist of 12-weeks of steady, continuous exercise (walking) performed with or without a treadmill 4 times a week. Each exercise session will consist of a 10-minute warm up where you will walk at an intensity that still allows you to carry on a conversation with someone. After the warm up, you will walk for 30-minutes at an intensity that makes it difficult for you to carry on a conversation. At the end of the session you will do a 10-minute cool down at the same intensity as the warm up. Every two weeks the time you spend at the intensity where you are unable to carry on a conversion will increase by 5-minutes, to a maximum of 75-minutes total (warm up – main exercise – cool down) by the end of week-12. Three of the 4 exercise sessions performed each week will be done at the R.J.D. Williams Building in the city of Saskatoon (221 Cumberland Ave North, Saskatoon, SK) and under the supervision of a certified exercise physiologist. For the 4th exercise session done each week, you have the option of either performing it at the R.J.D. Williams Building or you may perform your walking exercise at another location of your choice, with or without the use of a treadmill. If you choose to perform the 4th exercise session outside the R.J.D. Williams Building you will be asked to record your exercise on a training log that will be handed in at the end of the study. The Williams building is open Monday to Friday 8:00am to 10:00pm and on Saturday and Sunday from 8:00 am till 5:00pm. You and the researcher will determine a mutually convenient time based on the times listed above for you to come in and exercise

At three time points during the study, both the exercise group and non-exercise group will be measured on a number of different parameters. The specific testing time points are 1)

prior to the start of the intervention, 2) midway through the intervention (week-6) and 3) between 24 and 72 hours after the final exercise session (exercise group) or within 72-hours of the end of week-12 (non-exercise group). Please refer to the *Study Testing Procedures* heading below for further information on the measurements that will be conducted.

Photographs taken using a digital camera by Brittany Gadzosa will take place through out the study. The photos will be taken solely for research purposes (photos will be used in presentation of the data at conferences and symposiums) and all identification markers on participants will be blocked out (example: faces). If you do agree to have your photo taken, we ask that you sign the photo release form at the end of this consent form. If you do participate in the study, but do not want your photo taken, please do not fill out the photo release form.

Study Testing Procedures:

Both the exercise group and the non-exercise group will undergo all testing procedures that take place at three time points during the study. The testing procedures during the study will take place in the morning (between 7:00am and 10:00am) at specific laboratories (exercise physiology; phlebotomy; body composition) in the PAC after a 12-hour overnight fast. One testing procedure, (Dual-energy X-ray absorptiometry scan [DXA]), will take place in the evening at the R.J.D. Williams Building during the same week of testing at the PAC.

Each testing visit will be on **day 6 through 9 of your menstrual cycle** as leptin and kisspeptin levels do vary during your menstrual cycle. To ensure that you are tested during day 6 to 9 of your menstrual cycle, you will be asked to email Brittany Gadzosa (please see contact information at the top of the document) on the first day of menstruation, which will allow her to book you in for testing on the appropriate day.

The specific testing procedures will consist of a 24-hour dietary log, anthropometric measures (BMI calculation, waist girth measurement), a single blood draw, submaximal exercise test, a 7-day Physical Activity Questionnaire for adults (7-day PAQ-AD), a 3-day dietary recall (DR) and body composition assessment. You will need to wear comfortable clothing and walking/running shoes. At the end of the testing session you will be offered granola bars and juice boxes to raise your blood sugar to your pre 12-hour fast levels. These same tests will be conducted at three time points throughout the study; prior to the start of the study, mid-way through the study (week-6) and at the end of the study (within 72-hours after the completion of the last exercise session in week 12).

24- Hour Dietary Log

In the 24-hours before the first testing session you will be asked to fill out a dietary log to track all the food, liquids and vitamins or supplements you have taken. You will be provided with the dietary log sheets to fill out as well as a sheet helping you to estimate portion sizes of food. For the following two testing sessions you will be asked to eat the same amount of foods, liquids and supplements the 24-hours prior to testing that you did before the first testing session. You are being asked to replicate your diet the 24-hours prior to each testing session because leptin level can acutely change based on food ingested.

Anthropometric Measurements

Height will be taken at the nearest 0.5cm and weight will be taken to the nearest 0.1kg. Both these measurements will then be used to determine your BMI and any changes in it over the study. Also, a waist girth will be measured to the nearest 0.5cm, to record the change in tissue centered around your waist.

Blood Draw

You will have roughly 2 teaspoons (2 x 7 ml) of blood drawn from a single vein at the inside of your elbow by a needle. A trained professional will perform the blood draw. The concentration of each of the hormones of interest in the study (leptin, kisspeptin, ghrelin, adiponectin, POMC and NPY) will be measured from this blood sample.

Submaximal Aerobic Exercise Test

You will perform the Ebbeling Single Stage Treadmill Walking test to measure your cardiovascular fitness. You will warm up on a treadmill by walking for 4 minutes. After 4 minutes the speed will remain constant; however, the incline of the treadmill will increase by 5%. This will be similar to walking up a small hill. You will continue walking for 4 to 5 more minutes at this incline until the test has ended. Although improvement in fitness is not a main outcome measure, there is very little data on the relationship between the specific hormones tested in this study and on an individuals' fitness level, therefore this study provides the opportunity to collect data on changes in cardiovascular fitness and adipokines for further analysis.

7-Day Physical Activity Questionnaire for Adults (PAQ-AD) & 3-Day Dietary Recall (DR)

You will be asked to complete a 7-day PAQ-AD (similar to the 2-month PAQ-AD) and a 3-day DR to record your physical activity/exercise habits and dietary habits from the start to the end of the study. At this stage of the testing you will be offered juice boxes and granola bars to eat.

Body Composition Assessment

The DXA scan will take place in the evening at the Williams Building during the same week of the testing sessions. The DXA scan is a full body x-ray scan that measures an individual's percent fat mass and percent fat free mass. The risk of radiation from the DXA is no higher than what you experience on a transcontinental flight. An x-ray technician will perform the scan.

Summary of Time Commitment:

- Initial screening procedures: 90 minutes
- Testing sessions: 120 minutes x 1 first testing session; 90 minutes x 2^{nd} and 3^{rd} testing session throughout the study
- Exercise sessions (if randomized into exercise group): 50 hours total over 4 times a week for 12-weeks

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

If you choose to participate in this study, there may be direct and indirect benefits for you. If you are randomized into the exercise intervention group you will be able to exercise for 12-weeks under the guidance of a certified exercise physiologist. Continuous exercise has been shown to have beneficial effects including improved cardiovascular health (endurance). Also continuous exercise is known to decrease weight, the risk of type II diabetes, atherosclerosis and certain cancers.

If you are randomized into the non-exercise group you will be a given a 12-week pass to the PAC gym after the testing period of the study is completed. This will enable you to participate in "open" fitness classes, or use the exercise equipment in the Fit Center so that you,

too, will have an opportunity to achieve the positive benefits of participation in a physical activity program.

It is hoped the information gained from this study can be used in the future to benefit women who are above a healthy weight and are trying to conceive.

ARE THERE POSSIBLE RISKS AND DISCOMFORTS?

If you choose to participate in this study, there are possible risks and discomforts you may face. In relation to screening and testing procedures there is the possibility of feeling nauseous or light headed from the 12-hour fast the day prior to testing and due to blood draws. Juice boxes and granola bars will be offered at the end of each testing session to help raise blood sugar levels to pre 12-hour fast levels. Also, there is the risk of infection from the blood draws; however, this risk will be minimized by having all samples taken in a clean environment that has obtained all certifications from the University of Saskatchewan Biosafety committee or at the Royal University Hospital and done by a trained professional (phlebotomist/registered nurse). As well, there is a slight chance of bruising at the site of the blood draw.

To familiarize you with safe exercise habits and to introduce you to the specific exercise protocol you will be performing in the study, you will be asked to say for an extra 30 to 45-minutes on the first DXA scan test day (prior to start of intervention).

There is a small radiation exposure from the DXA machine, however research has demonstrated that one scan is equal to the amount of low dose radiation experienced on a single transcontinental flight. Finally, muscle soreness, muscle strains, ligament sprains or undiagnosed cardiac or pulmonary conditions could occur and/or become evident during the testing sessions or the exercise intervention.

WHAT IF NEW INFORMATION BECOMES AVAILABLE THAT MAY AFFECT MY DECISION TO PARTICIPATE?

During the course of this study, new information that may affect your willingness to continue to participate will be provided to you by the researcher immediately.

WHAT HAPPENS IF I DECIDE TO WITHDRAW?

Your participation in this research is voluntary. You may withdraw from this study at any time. You do not have to provide a reason. There will be no penalty or loss of benefits if you choose to withdraw.

If you choose to enter the study and then decide to later withdraw, all data collected about you during your enrolment **will be retained** for analysis. Also, you will be invited to come into the PAC and the R.D.J Williams Building to have one final blood draw (exit blood draw), height, weight, waist circumference measurements and DXA scan done. These measurements are being done to determine if there are any change between the hormones measured and changes in body weight by the time you withdraw from the study. You will be asked to follow all pre-testing procedures including the 12-hour overnight fast and being on day 6 to 9 of your menstrual cycle for the exit blood draw.

CAN I BE ASKED TO LEAVE THE STUDY?

You may be withdrawn from the study if staying in the study would be harmful, you need treatment not allowed in the study, you fail to follow instructions, **you become pregnant**, your menstruation cycle becomes equal or greater 35 days or the study is cancelled by the sponsor for administrative or other reasons. **If you do become pregnant or your menstrual cycle changes while in the study, you must alert the researcher as soon as possible.**

WILL I BE INFORMED OF THE RESULTS OF THE STUDY?

The results of the experiment will be provided to you at the end of study from Brittany Gadzosa. Brittany Gadzosa will either email or mail the results out to you (please see the consent form to check which delivery method you would prefer). All results will be written in general lay language and, if further explanation of the results is needed, please do not hesitate to contact Brittany Gadzosa.

The information will also be shared within the academic and general communities. Firstly the results will be published as the thesis project for Brittany Gadzosa. Also, Brittany Gadzosa and her committee aim to publish results from the study in scientific journals and present the findings at academic conferences. All data that is presented will be done so in a cumulative fashion and will not include any specific identifiers to individual participants.

WHAT WILL THE STUDY COST ME

You will not be charged for any research-related procedures. You will not be paid for participating in this study. You will not receive any compensation, or financial benefits for being in this study, or as a result of data obtained from research conducted under this study.

You will reimbursed for the cost of parking at the PAC and Royal University Hospital (initial screening session, HbA1c Test and 3 testing sessions). You will not be reimbursed for the cost of parking at the R.J.D. Williams Building as there is free street parking surrounded the building.

You will not be reimbursed for the cost of going to an exercise facility, if you choose to do your 4th exercise session each week at a location different from the R.J.D. Williams Building.

WHAT HAPPENS IF SOMETHING GOES WRONG?

If there is any emergency situation in any of the exercise or testing sessions, immediate medical care will be provided to you. By signing this document, you do not waive any of your legal rights.

In case of an emergency related to the study that happens outside of the exercise or testing session, please seek immediate medical care and notify the researcher at the 24-hour study phone number as soon as possible.

WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

In Saskatchewan, the Health Information Protection Act (HIPA) defines how the privacy of your personal health information must be maintained so that your privacy will be respected.

At the beginning of the study you will be provided with your study identification numeric code and it will be used on all documents related to the study in place of your name to keep your information confidential. The only time that your name and your study identification code will together is on a master sheet that will be stored in a separate location and in a locked cabinet compared to all other study documents.

However, please note that on the requisition form for the HbA1c test you will be asked to provide personal information such as name, date of birth, address, phone number and your Saskatchewan health care number. The Saskatoon Health Region requires this information, including your health care number, as this is how they keep track of their results and records. Only those directly involved with the study (Researchers listed in this form and the staff at the Royal University Hospital who will draw the blood or test the blood) will be in direct contact with your personal information listed on the requisition form. Since your official laboratory

results will contain personal identifying information, these documents will be stored with the master sheet to keep them separate from all documents containing only your unique study identification numeric code and to ensure your personal information does not become identifiable.

No information that discloses your identity will be released or published without your specific consent. Some authorities have a duty to check the study and records to make sure all the information is correct. The study and records may be inspected in the presence of the investigator or the University of Saskatchewan Ethics Board. If you decide to withdraw from this study, your records will be made available to the University of Saskatchewan Ethics Board. However, they will only look at your records up to the date of your withdrawal, except where the reporting of side effects associated with the study medication is required. Rarely, your study documents may be obtained by courts of law. You may ask the study doctor to see and copy your personal health information related to the study. You may also ask the study doctor to correct any study related information about you that is wrong.

All the records from the study will be kept for 5 years in a locked file cabinet and office at the PAC, after those 5 years all documents will be shredded in a manner that makes them unreadable.

The results of this study may be presented in a scientific meeting or published, but your identity will not be disclosed.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY?

If you have any questions or desire further information about this study before or during participation, you can contact *Brittany Gadzosa* (*bcg625@mail.usask.ca* or 306-966-1305 / 306-715-9643) or *Dr. Carol Rodgers* (carol.rodgers@usask.ca or 306-966-1061).

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Biomedical Research Ethics Board, at 306-966-2975 (out of town calls 1-888-966-2975). The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Research Ethics Board.

UNIVERSITY OF SASKATCHEWAN

CONSENT TO PARTICIPATE

Study Title: The Effects of a 12-Week Exercise Intervention on Circulating Adipokines, Metabolic and Reproductive Hormone Levels in Obese Women

- I have read (or someone has read to me) the information in this consent form.
- I understand the purpose and procedures and the possible risks and benefits of the study.
- I was given sufficient time to think about it.
- I had the opportunity to ask questions and have received satisfactory answers.
- I understand that I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my future relationships.
- I give permission to the use and disclosure of my de-identified information collected for the research purposes described in this form (written or via photograph)
- I understand that by signing this document I do not waive any of my legal rights.
- I understand I will be given a signed copy of this consent form.

Please check and initial the appropriate box to indicate your decision:

___Yes, I agree that you may inform my family doctor of my participation in this study

_____ *No, I do not want you to inform my family doctor of my participation in this study*

Please check and initial which delievery methods for obtaining results you would perfer:

_____ *Email* _____ *Mail*

I agree to participate in this study:

Printed name of participant: Signature Date

Printed name of person obtaining consent: Signature Date

UNIVERSITY OF SASKATCHEWAN

PHOTO RELEASE FORM

Study Title: The Effects of a 12-Week Exercise Intervention on Circulating Adipokines, Metabolic and Reproductive Hormone Levels in Obese Women

I hereby give permission for images of myself, captured during ***The Effects of a 12-Week Exercise Intervention on Circulating Adipokine, Metabolic and Reproductive Hormone Levels in Obese Women*** study through photo and digital camera to be used solely for the purpose of the ***presentation and publication of material from the study***, and waive any rights of compensation or ownership thereto.

Name of Participant (Please Print): _____

Signature of Participant: _____

Date: _____

Consent Form – Understanding Checklist

1) Understand purpose of study ☐
2) Family Dr. does not need to be notified but we can if you would like ☐
3) Inclusion Criteria ☐
    a. Must meet all to be included in study ☐
4) Study Phases ☐
    a. Screening Procedures ☐
        i. BMI & WC ☐
        ii. PARQ+ ☐
        iii. 2-month Physical Activity Question ☐
        iv. Menstrual Cycle History ☐
        v. Hemoglobin A1C Test ☐
    b. Exercise Intervention ☐
        i. Exercise Group ☐
        ii. Non-Exercise Group ☐
        iii. Randomization ☐
        iv. Exercising Outside ☐
        v. Orientation session ☐
    c. Testing x3 ☐
        i. Based on timing of menstrual cycles ☐
        ii. Pre – Mid – Post ☐
        iii. This means the exercise intervention will potentially go longer than three months ☐
        iv. Lead in procedures start the day before ☐
            1. Need to record all food you eat and its measurements – 24-hour food log ☐
            2. Fast 10 to 12 hours night before ☐
            3. Anthropometric Measures ☐
            4. Blood Draw ☐
            5. Submaximal Exercise Test ☐
            6. 2 questionnaires ☐
            7. DXA Scanning (Evening at Williams) ☐
5) Benefits of Participating ☐
6) Possible Risk and Discomfort ☐
7) Decide to withdraw ☐
8) Can you be asked to leave study ☐
9) Informed of the results of the study ☐
10) Confidentiality ☐

APPENDIX I: Physical Activity Readiness Questionnaire Plus (PAR-Q+)

CSEP approved Sept 12 2011 version

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

SECTION 1 - GENERAL HEALTH

	Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	☐	☐
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	☐	☐
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	☐	☐
5.	Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	☐	☐
7.	Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.

Go to Section 3 to sign the form. You do not need to complete Section 2.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow the Canadian Physical Activity Guidelines for your age (www.csep.ca/guidelines).
- You may take part in a health and fitness appraisal.
- If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist® (CSEP-CEP) or CSEP Certified Personal Trainer® (CSEP-CPT).
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the questions above, please GO TO SECTION 2.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.

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SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?		☐ If yes, answer questions 1a-1c	☐ If no, go to question 2
	1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/ or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
	1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.	Do you have Cancer of any kind?		☐ If yes, answer questions 2a-2b	☐ If no, go to question 3
	2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	☐	☐
	2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm		☐ If yes, answer questions 3a-3e	☐ If no, go to question 4
	3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial brillation, premature ventricular contraction)	☐	☐
	3c.	Do you have chronic heart failure?	☐	☐
	3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	☐	☐
	3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes		☐ If yes, answer questions 4a-4c	☐ If no, go to question 5
	4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	☐	☐
	4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	☐	☐
	4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	☐	☐
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)		☐ If yes, answer questions 5a-5b	☐ If no, go to question 6
	5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	5b.	Do you also have back problems affecting nerves or muscles?	☐	☐

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure		☐ If yes, answer questions 6a-6d	☐ If no, go to question 7
	6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
	6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	☐	☐
	6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia		☐ If yes, answer questions 7a-7c	☐ If no, go to question 8
	7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	☐	☐
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event		☐ If yes, answer questions 8a-c	☐ If no, go to question 9
	8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	8b.	Do you have any impairment in walking or mobility?	☐	☐
	8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?		☐ If yes, answer questions 9a-c	☐ If no, read the advice on page 4
	9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
	9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	☐	☐
	9c.	Do you currently live with two chronic conditions?	☐	☐

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

- You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise profesional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

SECTION 3 - DECLARATION

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- Please read and sign the declaration below:

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:
Canadian Society for Exercise Physiology
www.csep.ca

KEY REFERENCES
1. Jamnik VJ, Warburton DER, Makarski J, McKenzie DC, Shephard RJ, Stone J, and Gledhill N. Enhancing the eectiveness of clearance for physical activity participation; background and overall process. APNM 36(S1):S3-S13, 2011.
2. Warburton DER, Gledhill N, Jamnik VK, Bredin SSD, McKenzie DC, Stone J, Charlesworth S, and Shephard RJ. Evidence-based risk assessment and recommendations for physical activity clearance; Consensus Document. APNM 36(S1):S266-s298, 2011.

The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.

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APPENDIX J: Physician Physical Activity Readiness Clearance Form

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CSEP-PATH: PHYSICIAN PHYSICAL ACTIVITY READINESS CLEARANCE

Dear Physician, _____

Patient Name: _____

Date: _____

Your patient has consulted a Canadian Society for Exercise Physiology - Certified Personal Trainer® (CSEP-CPT) for a physical activity, fitness and lifestyle assessment and/or personal training services.

Although evidence demonstrates that becoming more active is very safe for most people and yields many health benefits, it is important to identify clients who may need a more thorough evaluation before doing a fitness assessment or becoming much more physically active.

During our standardized screening procedures we became aware that your patient:

☐ Answered "Yes" to one or more questions on the Physical Activity Readiness Questionnaire (PAR-Q+) – see copy attached. Specific concern:

_____

☐ Had a Resting Heart Rate of ____ (above the safety cut-off of 99 bpm)

☐ Had a Resting Blood Pressure of ____/____ (above the safety cut-off of 144/94 mmHg)

To ensure that your patient proceeds in the safest way possible, they were advised to consult with you about becoming more physically active. Please complete and sign this form, indicating any necessary physical activity restrictions, and have your patient return the form to their CSEP-CPT.

Based upon my review of the health status of _____ , I recommend:

☐ Unrestricted physical activity based on the *Canadian Physical Activity Guidelines* - start slowly and build up gradually

☐ Progressive physical activity:

- ☐ With avoidance of: _____
- ☐ With inclusion of: _____

☐ Only a medically-supervised exercise program until further medical clearance

☐ No physical activity

Physician Name (please print):

Signed: _____

Date: _____

Physician/Clinic Stamp:

If you have any questions regarding the physical activity, fitness and lifestyle assessment, the PAR-Q+, or the services provided by the CSEP-CPT, please contact:

CSEP-CPT: _____

Email and Phone: _____

NOTE: This Physician Physical Activity Readiness Clearance is valid for a maximum of one year from the date it is completed, and becomes invalid if your patient's medical condition worsens.

APPENDIX K: 2 Month Physical Activity Questionnaire (2 month PAQ-AD)

University of Saskatchewan

Effects of a 12-Week Exercise Intervention on Circulating Adipokine, Metabolic and Reproductive Hormone Levels in Obese Women

Physical Activity Questionnaire – Adults (PAQ-AD)
2-Month Recall

Participant ID #:_____ Date: _____

We are trying to find out about your level of physical activity from ***the last 7 days*** (in the last week). This includes activities that make you sweat, make your legs feel tired, or make you breathe hard, such as team sports, running, strenuous occupational activities, and others.

Remember:

1. There are no right and wrong answers — this is not a test.
2. Please answer all the questions as honestly and accurately as you can — this is very important.

---

1. Physical activity in your spare time: Have you done any of the following activities in the past 7 days (last week)? If yes, how many times? (Mark only one circle per row.)

	No	1-2	3-4	5-6	7 times or more
Rock climbing.....	❍	❍	❍	❍	❍
Rowing/canoeing	❍	❍	❍	❍	❍
Tennis/squash .....…	❍	❍	❍	❍	❍
Stair climber (or other similar equipment).....	❍	❍	❍	❍	❍
Walking for exercise	❍	❍	❍	❍	❍
Heavy yard work	❍	❍	❍	❍	❍
Jogging or running	❍	❍	❍	❍	❍
Bicycling .....……	❍	❍	❍	❍	❍
Aerobics (or other exercise class)...	❍	❍	❍	❍	❍
Swimming .....…	❍	❍	❍	❍	❍
Baseball, softball .....…	❍	❍	❍	❍	❍
Dance .....…	❍	❍	❍	❍	❍
Football	❍	❍	❍	❍	❍
Badminton .....…	❍	❍	❍	❍	❍
Soccer	❍	❍	❍	❍	❍
Street/floor hockey	❍	❍	❍	❍	❍
Volleyball	❍	❍	❍	❍	❍
Basketball .....….	❍	❍	❍	❍	❍
Skating (in-line/ice)	❍	❍	❍	❍	❍
Cross-country skiing .....…	❍	❍	❍	❍	❍
Ice hockey/ringette .....…	❍	❍	❍	❍	❍
Martial arts.....……	❍	❍	❍	❍	❍
Weight training.....…….	❍	❍	❍	❍	❍
Other:					
_____..…	❍	❍	❍	❍	❍
_____..…	❍	❍	❍	❍	❍

2. In the last 7 days, *during the morning,* how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity)? (Check one only.)

None ..... ❍
1 time last week ..... ❍
2 or 3 times last week ..... ❍
4 or 5 times last week..... ❍
6 or 7 times last week ..... ❍

3. In the last 7 days, *after lunch and before supper,* how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity)? (Check one only.)

None ..... ❍
1 time last week ..... ❍
2 or 3 times last week ..... ❍
4 or 5 times last week..... ❍
6 or 7 times last week..... ❍

4. In the last 7 days, *during the evening,* how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity)? (Check one only.)

None ..... ❍
1 time last week ..... ❍
2 or 3 times last week ..... ❍
4 or 5 last week ..... ❍
6 or 7 times last week ..... ❍

5. *On the last weekend*, how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity)? (Check one only.)

None ..... ❍
1 time ..... ❍
2 — 3 times ..... ❍
4 — 5 times ..... ❍
6 or more times ..... ❍

6. Which *one* of the following describes you best for the last 7 days? Read *all five* statements before deciding on the *one* answer that describes you.

A. All or most of my free time was spent doing things that involve little physical effort .....❍
B. I sometimes (1 — 2 times last week) did physical things in my free time (e.g. played sports, went running, swimming, bike riding, did aerobics) .....❍
C. I often (3 — 4 times last week) did physical things in my free time .....❍

D. I quite often (5 — 6 times last week) did physical things in my free time … ❍

E. I very often (7 or more times last week) did physical things in my free time ❍

7. Mark how often you did physical activity (for example: playing sports, exercise classes, strenuous occupational activity).

	None	Little bit	Medium	Often	Very often
Monday	❍	❍	❍	❍	❍
Tuesday	❍	❍	❍	❍	❍
Wednesday	❍	❍	❍	❍	❍
Thursday	❍	❍	❍	❍	❍
Friday	❍	❍	❍	❍	❍
Saturday	❍	❍	❍	❍	❍
Sunday	❍	❍	❍	❍	❍

8. Were you sick last week, or did anything prevent you from doing your normal physical activities? (Check one.)

Yes .....❍
No ..... ❍

If Yes, what prevented you? _____

9. Are the responses you made on the PAQ-AD representative of your physical activity behavior in the prior two months? (Circle one.)

YES NO

APPENDIX L: Self-report Menstrual & Medical History Questionnaire

Effects of a 12-week Exercise Intervention on Circulating Adipokine, Metabolic and Reproductive Hormone Levels in Obese Women

Participant ID #: _____ Date: _____

Self-Report Menstrual & Medical History Questionnaire

Instructions: Please circle the appropriate answer the pertains to you

1. Are you currently pregnant?

   YES NO

2. What is the average length of your menstrual cycle in days?
   Menstrual cycle is measured from the start of your period to the start of your next period
   _____

3. Have you ever experienced abnormal menstrual cycle lengths (greater than 35 days per cycle)?

   YES NO

4. Have you ever been diagnosed with a reproductive disorder such as, Polycystic Ovary Syndrome or Hypogonadism?

   YES NO

5. Are you currently taking any form of hormone contraception or hormone replacement therapy?

   YES NO

6. If you are **NOT** taking any form of hormone contraception or hormone replacement therapy, have you taken either within the past two months?

   YES NO

7. Have you been weight stable (have not lost or gained 2-3kg) within the prior 2 months?

   YES NO

8. Have you ever been diagnosed with one of the following (1) Insulin Resistance (2) Pre-diabetes (3) Type 1 diabetes (4) Type II Diabetes

YES NO

If yes, what were you diagnosed with? _____

9. Do you consider yourself to be a healthy women free of any past or current medical condition?

YES NO

University of Saskatchewan
Effects of a 12-Week Exercise Intervention on Circulating Adipokine, Metabolic and Reproductive Hormone Levels in Obese Women

24-Hour Dietary Log

Participant ID # _____ **Date: _____**

PLEASE LIST EVERY FOOD AND DRINK YOU ATE TODAY

Time	Food Item	Type & Preparation	Amount	Brand Name
Morning Example	***Cereal***	***Cornflakes***	***1 Cup***	***Kellogg***
	Milk	***1%***	***½ Cup***	***Dairyland***
Mid-Morning				
Noon Meal				

Midday				
Evening Meal				
Before Bed				

Was this intake usual? Circle one: YES NO
If **NO** explain why not:

_____

Did you take any vitamins or minerals during the day? Circle one: YES NO
If **YES**, please list:

_____

APPENDIX N: 7 day Physical Activity Questionnaire (7-day PAQ-AD)

University of Saskatchewan
Effects of a 12-Week Exercise Intervention on Circulating Adipokine, Metabolic and Reproductive Hormone Levels in Obese Women

Physical Activity Questionnaire – Adults (PAQ-AD)
7-Day Recall

Participant ID #:_____ Date:_____

We are trying to find out about your level of physical activity from ***the last 7 days*** (in the last week). This includes activities that make you sweat, make your legs feel tired, or make you breathe hard, such as team sports, running, strenuous occupational activities, and others.

Remember:

1. There are no right and wrong answers — this is not a test.
2. Please answer all the questions as honestly and accurately as you can — this is very important.

---

1. Physical activity in your spare time: Have you done any of the following activities in the past 7 days (last week)? If yes, how many times? (Mark only one circle per row.)

	No	1-2	3-4	5-6	7 times or more
Rock climbing.....	❍	❍	❍	❍	❍
Rowing/canoeing	❍	❍	❍	❍	❍
Tennis/squash	❍	❍	❍	❍	❍
Stair climber (or other similar equipment).....	❍	❍	❍	❍	❍
Walking for exercise	❍	❍	❍	❍	❍
Heavy yard work	❍	❍	❍	❍	❍
Jogging or running	❍	❍	❍	❍	❍
Bicycling	❍	❍	❍	❍	❍
Aerobics (or other exercise class)...	❍	❍	❍	❍	❍
Swimming	❍	❍	❍	❍	❍
Baseball, softball	❍	❍	❍	❍	❍
Dance	❍	❍	❍	❍	❍
Football	❍	❍	❍	❍	❍
Badminton	❍	❍	❍	❍	❍
Soccer	❍	❍	❍	❍	❍
Street/floor hockey	❍	❍	❍	❍	❍
Volleyball	❍	❍	❍	❍	❍
Basketball	❍	❍	❍	❍	❍
Skating (in-line/ice)	❍	❍	❍	❍	❍
Cross-country skiing	❍	❍	❍	❍	❍
Ice hockey/ringette	❍	❍	❍	❍	❍
Martial arts.....	❍	❍	❍	❍	❍
Weight training.....	❍	❍	❍	❍	❍
Other:					
_____.....	❍	❍	❍	❍	❍
_____.....	❍	❍	❍	❍	❍

2. In the last 7 days, *during the morning*, how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity)? (Check one only.)

None ..... ❍
1 time last week ..... ❍
2 or 3 times last week ..... ❍
4 or 5 times last week..... ❍
6 or 7 times last week ..... ❍

3. In the last 7 days, *after lunch and before supper*, how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity)? (Check one only.)

None ..... ❍
1 time last week ..... ❍
2 or 3 times last week ..... ❍
4 or 5 times last week..... ❍
6 or 7 times last week..... ❍

4. In the last 7 days, *during the evening,* how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity)? (Check one only.)

None ..... ❍
1 time last week ..... ❍
2 or 3 times last week ..... ❍
4 or 5 last week ..... ❍
6 or 7 times last week ..... ❍

5. *On the last weekend*, how often were you very active (for example: playing sports, exercise classes, strenuous occupational activity)? (Check one only.)

None ..... ❍
1 time ..... ❍
2 — 3 times ..... ❍
4 — 5 times ..... ❍
6 or more times ..... ❍

6. Which *one* of the following describes you best for the last 7 days? Read *all five* statements before deciding on the *one* answer that describes you.

   F. All or most of my free time was spent doing things that involve little physical effort ..... ❍

   G. I sometimes (1 — 2 times last week) did physical things in my free time (e.g. played sports, went running, swimming, bike riding, did aerobics) ..... ❍

   H. I often (3 — 4 times last week) did physical things in my free time ..... ❍

   I. I quite often (5 — 6 times last week) did physical things in my free time ❍

   J. I very often (7 or more times last week) did physical things in my free time ❍

7. Mark how often you did physical activity (for example: playing sports, exercise classes, strenuous occupational activity).

	None	Little bit	Medium	Often	Very often
Monday	❍	❍	❍	❍	❍
Tuesday	❍	❍	❍	❍	❍
Wednesday	❍	❍	❍	❍	❍
Thursday	❍	❍	❍	❍	❍
Friday	❍	❍	❍	❍	❍
Saturday	❍	❍	❍	❍	❍
Sunday	❍	❍	❍	❍	❍

8. Were you sick last week, or did anything prevent you from doing your normal physical activities? (Check one.)

   Yes ..... ❍
   No ..... ❍

   If Yes, what prevented you? _____

APPENDIX O: 3 day Dietary Recall

University of Saskatchewan

Effects of a 12-Week Exercise Intervention on Circulating Adipokine, Metabolic and Reproductive Hormone Levels in Obese Women

3-Day Dietary Recall

Participant ID # _____ **Date: _____**

PLEASE LIST EVERY FOOD AND DRINK YOU ATE IN THE PAST 3 DAYS

DAY 1

DATE: _____

Time	Food Item	Type & Preparation	Amount	Brand Name
Morning Example	***Cereal***	***Cornflakes***	***1 Cup***	***Kellogg***
	Milk	***1%***	***½ cup***	***Dairyland***
Mid-Morning				
Noon Meal				

Midday				
Evening Meal				
Before Bed				

Did you take any vitamins or minerals during the day? Circle one: YES NO
If **YES**, please list:

DAY 2

DATE:_____

Time	Food Item	Type & Preparation	Amount	Brand Name
Morning				
Mid-Morning				
Noon Meal				
Midday				

Evening Meal				
Before Bed				

Did you take any vitamins or minerals during the day? Circle one: YES NO
If **YES**, please list:

DAY 3

DATE: _____

Time	Food Item	Type & Preparation	Amount	Brand Name
Morning				
Mid-Morning				
Noon Meal				
Midday				

Evening Meal				
Before Bed				

Did you take any vitamins or minerals during the day? Circle one: YES NO
If **YES**, please list:

Was your food and supplement intake over the past 3 days considered usual? Circle one:

YES NO

If **NO** explain why not:

_____

APPENDIX P: Randomization Sheet

1=Exercise Group
2=Control Group

Print Download in Excel Close

Research Randomizer Results

12 Sets of 2 Unique Numbers Per Set
Range: From 1 to 2 -- Unsorted

Job Status: **Finished**

Set #1:

p1=**1**, p2=**2**
R28 SB4

Set #2:

p3=**2**, p4=**1**
JB1 AM3

Set #3:

p5=**1**, p6=**2**
JA6 TR16

Set #4:

p7=**2**, p8=**1**
CJ13 IK12

Set #5:

p9=**1**, p10=**2**
JA15 CL11

Set #6:

p11=**2**, p12=**1**
LD19 VD4

Set #7:

p13=**2**, p14=**1**
RF24 AP22

Set #8:

p15=**2**, p16=**1**
FJ05

Set #9:

p17=**2**, p18=**1**

Set #10:

p19=**1**, p20=**2**

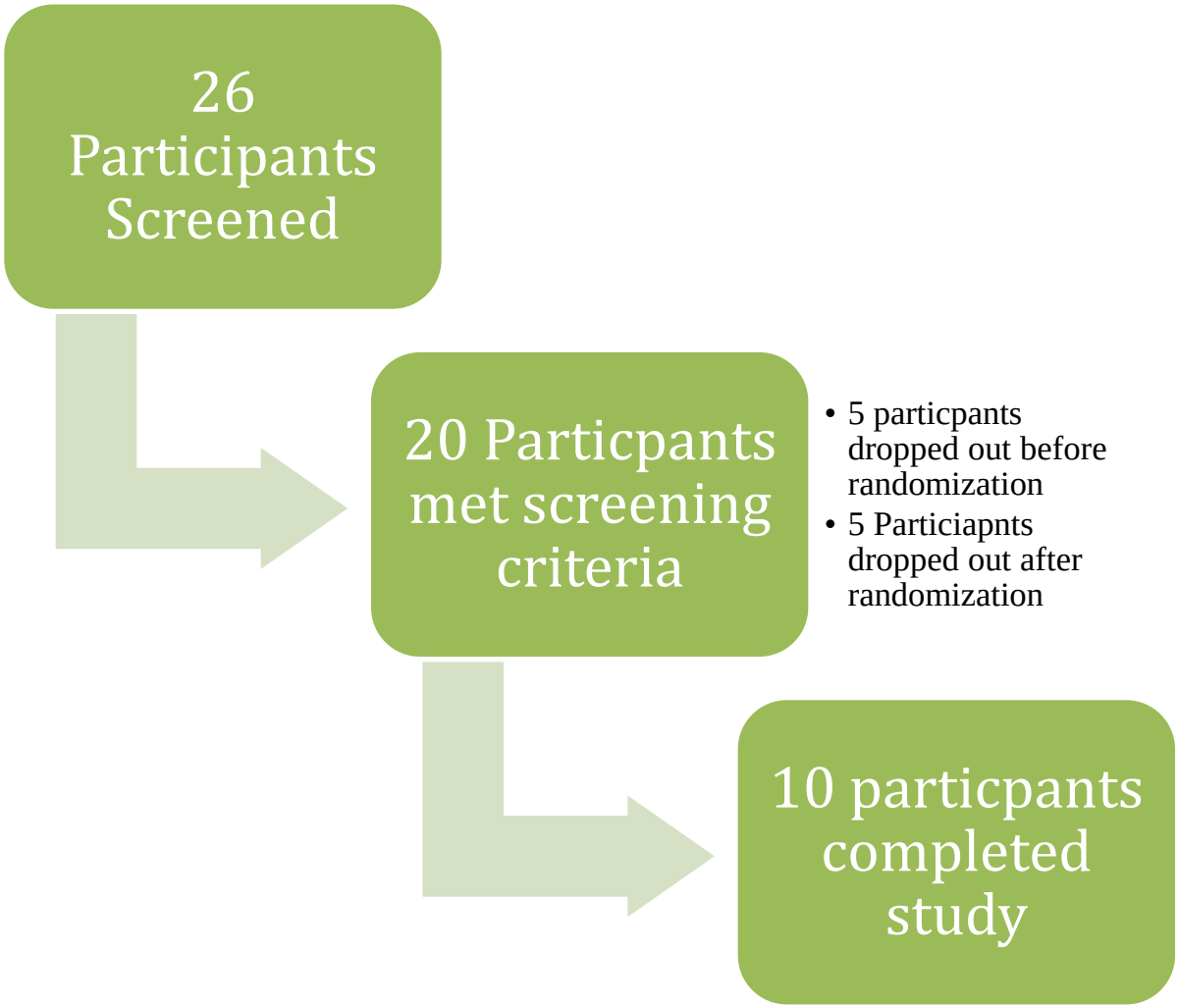
Set #11:

p21=**1**, p22=**2**

Set #12:

p23=**1**, p24=**2**

APPENDIX Q: Study Recruitment Flow Chart



APPENDIX R: Raw Leptin Levels

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Toll Free: (800) 988-1205
Fax: (650) 558-1686

Peptide Level Determination
Sample Assay Report

CLIENT:

Name:	Brittany Gadoza
Institution:	University of Saskatchewan
Address:	A124 Dept. of Pharmacology 107 Wiggin Road Saskatchewan, Saskatchewan S7N 5E5 Canada
Telephone:	
Email:	bcg625@mail.usask.ca

CONTACT:

Name:	Crystal Chang
Institution:	Phoenix Pharmaceuticals, Inc
Address:	330 Beach Road Burlingame, CA 94010
Telephone:	650-558-8898
Email:	crystalc@phoenixpeptide.com

Number of samples: 30

Type of sample: human plasma

Kit(s) used: Catalog# EK-003-12, Leptin (human) ELISA Kit

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Sample ID	Absorbance Value (OD)	Mean Result (ng/ml)	CV%	Dilution Factor	Adjusted Result (ng/ml)
AM3 (T1A)	0.757 0.734	2.94	1.9	40	117.5
AM3 (T2A)	0.686 0.671	2.71	1.4	20	54.1
AM3 (T3A)	0.835 0.862	3.29	2.0	20	65.7
SB4 (T1A)	0.803 0.840	3.20	2.8	40	127.8
SB4 (T2A)	1.773 1.824	6.32	1.7	20	126.4
SB4 (T3A)	0.644 0.619	2.54	2.4	40	101.7
JA6 (T1A)	0.689 0.635	2.65	5.0	20	53.0
JA6 (T2A)	0.694 0.694	2.76	0.0	10	27.6
JA6 (T3A)	0.669 0.647	2.63	2.1	20	52.7
R28 (T1)	1.148 1.171	4.31	1.2	20	86.3
R28 (T2)	1.078 1.056	4.01	1.3	20	80.2
R28 (T3)	0.702 0.684	2.76	1.6	40	110.2
CL11 (T1A)	0.923 0.972	3.62	3.2	20	72.4
CL11 (T2A)	1.094 1.156	4.20	3.4	20	84.0
CL11 (T3A)	1.061 0.925	3.77	8.4	20	75.4
CT13 (T1A)	1.099 1.030	4.00	4.0	40	160.2
CT13 (T2A)	1.960 1.914	6.74	1.5	20	134.8
CT13 (T3A)	0.985 0.918	3.63	4.3	40	145.2
JA15 (T1A)	0.923 0.966	3.61	2.8	20	72.2
JA15 (T2A)	0.904 0.901	3.47	0.2	20	69.4

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Sample ID	Absorbance Value (OD)	Mean Result (pg/ml)	CV%	Concentration Factor	Adjusted Result (pg/ml)
JA15 (T3A)	0.902 0.899	3.46	0.2	10	34.6
V417 (T1A)	1.152 1.083	4.18	3.8	20	83.5
V417 (T2A)	0.845 0.804	3.21	3.1	20	64.1
V417 (T3A)	0.618 0.649	2.55	3.0	40	101.9
RF24 (T1A)	1.079 1.093	4.07	0.8	20	81.5
RF24 (T2A)	1.183 1.189	4.40	0.3	20	88.0
RF24 (T3A)	1.141 1.099	4.19	2.3	20	83.7
FT25 (T1A)	0.737 0.774	2.97	3.0	40	118.8
FT25 (T2A)	1.329 1.416	5.00	3.9	20	99.9
FT25 (T3A)	1.435 1.479	5.26	1.9	20	105.2

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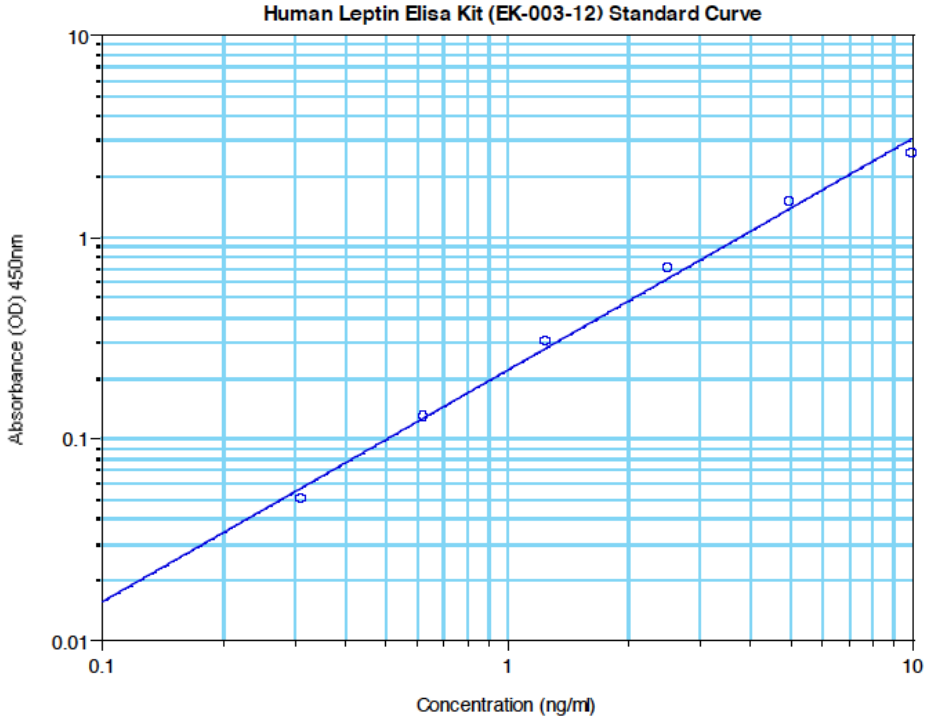

Log-Log Fit: Log(y) = A + B * Log(x):	A	B	R^2
Std (Standards: Concentration vs MeanValue)	-0.665	1.15	0.994

Positive Control

Sample	O.D.	Mean Result (ng/ml)	CV%
PC	0.219	0.967	6.5
	0.197		

APPENDIX S: Raw KiSS Data

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Peptide Level Determination
Sample Assay Report

CLIENT:

Name:	Brittany Gadoza
Institution:	University of Saskatchewan
Address:	A124 Dept. of Pharmacology 107 Wiggin Road Saskatchewan, Saskatchewan S7N 5E5 Canada
Telephone:	
Email:	bcg625@mail.usask.ca

CONTACT:

Name:	Crystal Chang
Institution:	Phoenix Pharmaceuticals, Inc
Address:	330 Beach Road Burlingame, CA 94010
Telephone:	650-558-8898
Email:	crystalc@phoenixpeptide.com

Number of samples: 30

Type of sample: human plasma

Kit(s) used: Catalog# FEK-048-56, Kisspetin-10 Fluorescent EIA Kit

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Sample ID	Absorbance Value (RFU)	Mean Result (pg/ml)	CV%	Concentration Factor	Adjusted Result (pg/ml)
AM3 (T1A)	2440.7 2291.2	7.14	4.5	4	1.78
AM3 (T2A)	2343.4 2217.5	8.62	3.9	4	2.15
AM3 (T3A)	2331.0 2327.5	7.64	0.1	4	1.91
SB4 (T1A)	2302.1 2437.2	7.06	4	4	1.76
SB4 (T2A)	2148.5 2218.5	10.65	2.3	4	2.66
SB4 (T3A)	2435.5 2360.5	6.57	2.2	4	1.64
JA6 (T1A)	2183.9 2134.1	11.23	1.6	4	2.81
JA6 (T2A)	2365.8 2562.7	5.77	5.6	2	2.88
JA6 (T3A)	2337.2 2263.8	8.18	2.3	4	2.05
R28 (T1)	2020.3 2100.8	14.09	2.8	4	3.52
R28 (T2)	2100.0 2195.6	11.57	3.1	4	2.89
R28 (T3)	2181.7 2148.1	11.07	1.1	4	2.77
CL11 (T1A)	2004.8 2384.0	11.34	12.2	4	2.84
CL11 (T2A)	2074.3 2032.0	14.28	1.5	4	3.57
CL11 (T3A)	1999.1 2038.8	15.44	1.4	4	3.86
CT13 (T1A)	2717.0 2786.0	7.28	1.8	4	1.82
CT13 (T2A)	2659.5 2630.1	8.74	0.8	4	2.19
CT13 (T3A)	2646.3 3201.8	5.96	13.4	4	1.49
JA15 (T1A)	2819.1 2673.2	7.39	3.8	4	1.85
JA15 (T2A)	2524.3 2553.1	10.49	0.8	4	2.62

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Sample ID	Absorbance Value (OD)	Mean Result (pg/ml)	CV%	Concentration Factor	Adjusted Result (pg/ml)
JA15 (T3A)	2498.0 2524.1	11.01	0.7	4	2.75
V417 (T1A)	2471.4 2476.6	11.73	0.1	4	2.93
V417 (T2A)	2313.3 2390.2	14.55	2.3	4	3.64
V417 (T3A)	3017.1 2960.3	4.79	1.3	4	1.20
RF24 (T1A)	2403.1 2632.4	11.10	6.4	4	2.77
RF24 (T2A)	2882.5 2930.6	5.54	1.2	2	2.77
RF24 (T3A)	2485.8 2564.8	10.76	2.2	4	2.69
FT25 (T1A)	2248.1 2357.6	15.90	3.4	4	3.98
FT25 (T2A)	2993.3 3003.5	4.70	0.2	2	2.35
FT25 (T3A)	3469.9 3413.7	1.95	1.2	2	0.98

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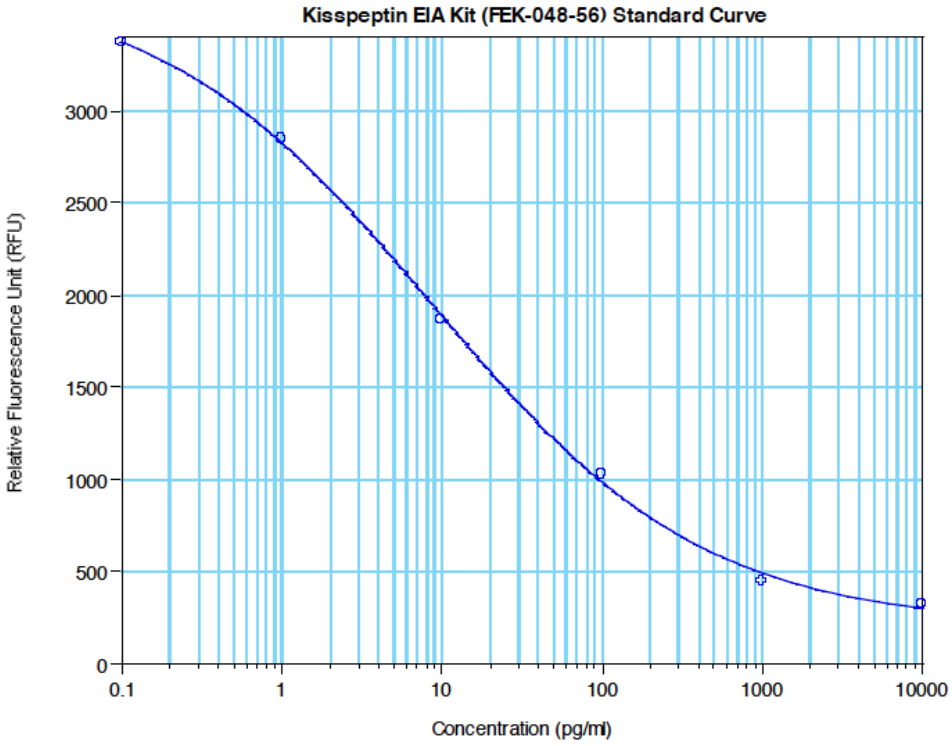

4-P Fit: y = (A - D)/(1 + (x/C)^B) + D:	A	B	C	D	R^2
STD (Standards: Concentration vs MeanValue)	3.7e+03	0.509	8.72	206	0.999

Weighting: Fixed

ED50: 8.72 pg/ml
Linear Range: 1.1 - 112 pg/ml